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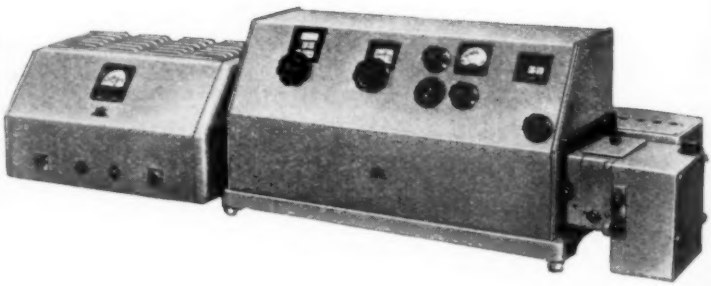

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TRAINING FOR MANAGEMENT IN BRITAIN

WHATEVER may result from the restrictive measures prescribed by the Chancellor of the Exchequer, Mr. Selwyn Lloyd, in his July budget, the occasion may prove to be one of historical significance. A party which has always adopted a pragmatical approach to politics has now accepted the principle that, in future, a real measure of planning is necessary in Britain to manage the affairs of State and to build up a balanced economy.

Britain's long-term planning must be concerned with two main problems. The first is how to inject enough capital into the right kinds of industry to produce a steadily expanding economy; the second is to produce an increasingly educated work-force to expand technology and apply it where it leads to the most beneficial results for the nation. While, as Prof. Allen has shown[1], successive Governments have been cautious about "the active promotion of development" until very recent times, the Chancellor's recent statement implies that the Government now recognizes that *laissez-faire* is no longer enough and that it must assume more direct responsibility for the promotion of industrial growth.

Investment policy will be mainly concerned with shifting the emphasis from traditional-based industries, such as shipbuilding and building, to science-based industries like the electrical, aircraft, chemical and man-made fibres organizations. Here the problem is one of acceleration, and much will depend on the courage of Ministers who will be required to inject capital into wealth-producing industries and withhold it from those which are in decline. Evidence of former service to the nation should no longer be sufficient to merit propping-up, for, as Allen says, "firms that are content to follow old paths for long periods cannot normally hope to survive in a dynamic economic system". The mark of a successful industry is its ability to accept and promote change, and there is little doubt that in recent years progress in Britain has not compared favourably with that of several foreign countries. Although the danger of technological unemployment seems to have been largely eliminated, certain industries, such as shipbuilding, building and printing, have shown marked obstruction to the introduction of improved methods and new techniques.

For this, Prof. Allen believes management must bear some responsibility. Many would share this view and would add that, for far too long, Government policy—relying on exhortation and leaving the direction of much of industry to management traditionalists—has served not only to ignore the need for promoting technological change but, in addition, has failed to provide a climate in which a more scientific management could grow. If Britain is accepted as a member of the European Economic Community, there will be need for much more co-ordinated economic planning and for Government support to produce managers who are adequately trained in business affairs.

The Chancellor has already announced some of the measures he proposes to take which will give more pointed direction to where and how quickly industrial expansion should take place. But unless the men and women are produced who can command and control this industrial growth, much of the planning will be frustrated. A Government lead to speed up the application of scientific thinking in industry is long overdue and is one which should now be forthcoming. In their survey of management throughout the industrial world, Prof. Harbison and Prof. Myers have shown how badly Britain prepares its management, compared with rapidly developing countries in Europe, Asia and America[2]. McGivering, Matthews and Scott[3], in one of the few systematic studies of the quality of management in Britain, suggest that not only are many British industries traditionally based but also that many of the managements which control them are trained by a traditional system no longer satisfactory. For this they attach some blame to a class system, based on excessive recruitment from 'public schools' and from 'Oxbridge'. McGivering, Matthews and Scott would readily support the attempt by industry to recruit the more intelligent products of the public schools as potential managerial recruits; the all-too-common practice which industry adopts of providing inducements for boys who, by grammar school standards, would be written off as failures, is regretted by them and many others. The recruitment of such products has done considerable damage to labour relations and has continued to depress the level of already low standards of management.

Economic planning for industrial growth should be accompanied by over-all development of the labour resources of Britain with the provision of opportunities for allowing the most able individuals to rise to the top. Those familiar with large-scale industry will know that, at present, this is far from true, and that technologists and men of science are particularly liable to frustration; too much of industry is still tightly controlled by remote financiers who rely on local rule-of-the-thumb managements to run their factories. Such control provides little stimulus for the application of scientific methods and procedures, and may explain much of the stagnation in industry from which Britain has so long suffered. In their studies of the kind of higher education facilities which Britain needs, it is essential that the Robbins Committee should pay attention not only to the academic training of scientists and technologists for industry but also to the education of them and others as potential managers.

Despite the untiring efforts of Lyndall F. Urwick, it is taking Britain a long time to realize that industry will not prosper until it builds up a professional body

of management. Each productivity team that visited the United States ten years ago reported on that country's greater use of mechanized power, and on the quality of management which had been responsible for the introduction of labour-saving techniques. Urwick[4] reminds us that the United States has been experimenting with education for business management for half a century. There are more than 1,000 institutions giving higher education in this field, and by 1970 it is estimated that there will be 600,000 students at university-level in this one subject alone. Developments now taking place at Cambridge, Edinburgh, Bristol, Leeds and other universities and technical institutions seem pitifully inadequate compared with standards in the United States and with what is being done in countries such as Germany, Japan, Israel, Switzerland, Canada and Australia. The responsibilities which confront British universities to provide suitable facilities for developing the managers of the next decade have been well put in a report which was commissioned by the Ford Foundation: "The issue is not a simple choice between liberal education and business education. The issue is how to combine both. This American experience is at Great Britain's disposal. Why should we hesitate to use it ?" In his new appointment as director of the British Institute of Management, perhaps the greatest service John Marsh can offer to British management is to persuade universities such as Manchester, Liverpool, Sheffield and Birmingham that there is a body of management principles which can be taught and that universities have an important part to play in this.

In teaching these principles, one of the cardinal points to be instilled into all prospective managing directors is the need for a clearly defined personnel policy for every industrial undertaking. This has been effectively argued by Guy Hunter[5], who shows very convincingly that, despite the thunder of the popular Press, banter with shop stewards and persistent asperities near the shop floor (which do exercise foremen and junior managers) are not the real points of difficulty for the vast number of higher management. "It is", he writes, "the co-ordination of specialists, the provision for future management, the recruitment and training of managers and the deployment and handling of the whole managerial group which is at present seen as critical". Crude and imperfectly understood as they are, these changes represent an encouraging advance from warfare to diplomacy, and underline the need for closer analysis of the structure and functioning of the industrial group. In this analysis the means of motivating industrial workers will need considerable investigation. Recent work carried out in the United States[6] has indicated that money and fringe benefits are no longer the prime motivators that they were; the needs they are designed to fill are now reasonably well met. If this is the case, industry must look to other spring-boards for action. These will be largely found in providing opportunities for self-realization, for achieving responsibility and for commitment in the affairs of the undertaking.

What kind of self-esteem can be achieved by individuals in organizations where the main objective is to get through to-day, where training facilities are non-existent, and where there is little thought for the morrow ? Such organizations still make up the mass of British industry and are so because, in general, successive Governments have been content to leave the development of training in industry to the good sense of employers and leading trade unionists. Despite the setting up of the Industrial Training Council, this development has not been forthcoming. If the Chancellor of the Exchequer wants industry to develop in the most profitable way, he must convince existing managements that they can no longer escape their responsibilities for providing adequate training facilities for young workers, for those who are displaced and need to seek other employment, for those who need re-training, as well as for supervisory staff. Through the Ministry of Labour much closer control must be exercised over what is being done, and much more direction should be given to help those who are unwilling to help themselves. Economic planning will be ineffective unless it is accompanied by a wind of change inside industry itself. Training should be used to develop new attitudes and skills; new systems and methods will follow.

[1] Allen, G. C., *The Structure of Industry in Britain: a Study of Economic Change* (Longmans, Green and Co., Ltd., London, 1961).
[2] Harbison, F., and Myers, C. A., *Management in the Industrial World* (McGraw-Hill Publishing Co., Ltd., London, 1961).
[3] McGivering, T., Matthews, D., and Scott, W. H., *Management in Britain* (Liverpool University Press, 1961).
[4] Urwick, L. F., *The Manager* (August, 1961).
[5] Hunter, G., *Studies in Management* (University of London Press, Ltd., London, 1961).
[6] Herzberg, F., *The Motivation of Work* (John Wiley and Sons, Inc., New York and London, 1959). McGregor, D., *The Human Side of Enterprise* (McGraw-Hill Book Co., Inc., New York, 1960).

X-RAY MICROSCOPY

X-ray Microscopy

By Dr. V. E. Cosslett and Dr. W. C. Nixon. (Cambridge Monographs on Physics.) Pp. xiv + 406 + 32 plates. (Cambridge: At the University Press, 1960.) 80*s*. net.

DURING the past decade the field of X-ray microscopy and its various analytical modifications has undergone a rapid development. One may ask, what is the use of X-ray microscopy when powerful optical and electron-optical magnifying systems are available which can give a linear resolution from the macroscopic level down to macromolecular dimensions. Certainly the present methods of X-ray microscopy cannot compete with the electron microscope with regard to resolving power. In fact, for practical purposes, the resolution of the present X-ray microscopic techniques is of the same order as that of the best optical microscope. It is true that under special conditions the resolving power of certain X-ray microscopic techniques might be somewhat better than that of the optical microscope, but it reaches by no means the high resolution of the electron microscope.

The great merits of the X-ray microscopic procedures are rather the analytical possibilities than a high resolution. The relatively simple and theoretically predictable interactions between X-rays and

material make it possible by X-ray microscopic procedures to extract information about chemical composition and crystallographic structure of localized areas in a microscopic specimen. It is with these applications in mind that Drs. V. E. Cosslett and W. C. Nixon have composed their monograph *X-Ray Microscopy*, published in the series "Cambridge Monographs on Physics", by Cambridge University Press. In their presentation the authors have had excellent sources of material, such as those which have appeared in the proceedings of two international symposia on X-ray microscopy and X-ray micro-analysis held in Cambridge during 1956 and Stockholm 1959, respectively. A third symposium of this kind is planned at Stanford University, California, 1962. But besides a very careful recording of the literature in the field of X-ray microscopy, the book contains unpublished observations from the authors' own laboratory.

The techniques for the production of X-ray images by contact microradiography, projection X-ray microscopy, reflexion X-ray microscopy and some other imaging procedures are well covered and together with the chapter on the production of X-rays a reader, both the physicist and the biologist, can get an advanced knowledge of the field.

The physics of X-ray microscopy differs from that of common X-ray physics, such as that used in X-ray diagnosis or X-ray therapy in that soft and very soft X-rays have to be used. At present, unfortunately, the numerical values of the X-ray constants, for example, the mass-absorption coefficients, for these soft X-rays, 1–50 Å., are not known with such a high precision as is desired, and in many cases no actual measurements are available at all and therefore only calculated values can be given. It is to be hoped that with the increasing use of X-ray micro-analytical procedures also the X-ray constants in the soft and very soft X-ray regions will be experimentally determined by precision measurements.

The monograph covers the three main forms of analytical applications of X-ray microscopy, namely, absorption micro-analysis, emission micro-analysis and micro-diffraction procedures. The techniques for specimen preparation are given both for inorganic and organic material, and an extensive presentation is devoted to X-ray microscopy of biological material.

It is clear that the possibilities of applying X-ray microscopic procedures to a variety of research problems now are fully established. Among the merits of these non-destructive techniques can be mentioned that it is possible to determine, for example, water and dry weight in a localized volume of a biological specimen less than one cubic micron in size. The quantities determined are in the order of 10^{-12}–10^{-15} gm., and the analytical error is in the order of 5 per cent. In the field of mineralogy and metallurgy the X-ray microscopic procedures, especially the scanning microprobe techniques for elementary analysis by emission, have proved to be most powerful tools. The same can be said about the micro-diffraction procedures; it has been possible to record diffraction patterns of crystals only about 1μ in diameter by such X-ray optical methods.

The field of X-ray microscopy and X-ray micro-analysis is rapidly expanding, and the book produced by Dr. Cosslett and Dr. Nixon is highly recommended to all those who have problems in analysing very small volumes of specimen, whether these workers are active in mineralogy, metallurgy or in organic chemistry, biology or medicine. ARNE ENGSTRÖM

MANUAL OF BACTERIA AND ACTINOMYCES

Diagnostik der Bakterien und Actinomyceten
Von Prof. N. A. Krassilnikov. Pp. xi+813. (Jena: Gustav Fischer Verlag, 1959.) 59 D.M.

THE distinguished Soviet author of this manual of bacteria and actinomyces bases his classification on the following four criteria: (1) phylogenesis, and in particular his own view of this inspired guesswork; (2) morphological characteristics; (3) habitat; (4) variability under experimental conditions.

In accordance with his phylogenetic point of view there are four classes of micro-organisms, namely, Actinomycetes, Eubacteriae, Myxobacteriae and Spirochaetae, all of which have been elevated from Orders within Class II Schizomycetes of Bergey's *Classification* (seventh edition). The four criteria for classification have resulted in a far-reaching re-arrangement of orders, families and even species within the families, at the same time excluding conceptions such as sub-orders and tribes. The international principles of taxonomy are largely disregarded.

Keys are provided for the orders, families and species which make it possible to find and identify a particular species within this system if one knows what one is looking for. However, if doubt exists about some characteristic of a certain species this *Diagnostic of Bacteria and Actinomycetes* is of little help and even often misleading.

To quote some examples, the diphtheria bacillus can be found in the class of Actinomycetes, the order of Mycobacteriales, the family of Mycobacteriaceae and the genus *Mycobacterium*, and it figures as a species together with *M. phlei*, *M. tuberculosis* and *M. leprae*. Of the latter it is stated that cultures grow on blood agar plates, broth, serum and substrates containing glycerin. Disregarding the completely erroneous statement on the culture of *M. leprae*, it appears that the reasons for including corynebacteria in the genus *Mycobacterium* are: branching, which, according to the author, occurs rarely and only on certain media and under certain conditions of growth, and marked sensitivity to the antibiotic streptomycin. Organisms hitherto known within the separate genera *Escherichia*, *Salmonella*, *Shigella*, *Haemophilus*, *Pasteurella*, *Brucella*, *Bordetella*, *Klebsiella*, *Aerobacter*, *Proteus* appear merely as species of the genus *Bacterium* within the class of Eubacteria. To quote an instance of inadequacy, the *X* and *V* requirements of hæmophili are not mentioned though it is stated in the case of *Haemophilus influenzae* that growth is only obtained "in the presence of serum or animal tissues, and that additional growth factors are required". "*H. parainfluenzae* cannot be distinguished from *H. influenzae* as regards morphology, culture and physiology". In our present state of reasonably advanced knowledge the task of writing a reliable systematic of microbiology appears to be beyond the capabilities of any single person, which is probably the reason for such glaringly erroneous statements. For future editions Krassilnikov would do well to extend consultation of a few colleagues in a few fields of microbiology to collaboration with many experts in many fields. In mitigation the author's statement in the preface must be quoted that "the work is intended mainly for bacteriologists working in general, agri-

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cultural, soil, water and industrial microbiology". Yet, this statement does not make it any easier to diagnose, with the help of this manual, a strain belonging to, say, the hæmophili.

Krassilnikov would be perfectly justified in using his views on phylogenesis, morphology and habitat as a basis for his classification, but most experienced microbiologists in the West would think it quite wrong to make experimental variability and all that that term implies a criterion for classification. Thus the presence of an antibiotic in a culture may lead to bizarre forms which, if taken into account, will distort the criterion of morphology: and how, for example, is a freshly isolated strain of *M. tuberculosis* resistant to streptomycin to be placed in this scheme of things? In the last analysis the author's fourth criterion is the fundamental difficulty for a microbiologist with Western training. It appears that 'Michurinism' is responsible for this divergence of outlook in view of Krassilnikov's statement that external factors are capable of causing changes of the living substance of micro-organisms while denying that any such changes are governed by genes.

The book is well produced and printed, with 285 figures, including photographs of cultures, line drawings and photomicrographs, the latter of inferior standard. The translation from the Russian is competently done on the whole though quite a number of errors have crept in due to the transcription of the names of Western authors from the phonetic spelling in the Russian original text. There are 33 pages of indexes to literature and 53 to organisms.

K. Zinnemann

SATELLITE AND SPACE ORBITS

Space Trajectories

A Symposium sponsored by the American Astronautical Society, the Advanced Research Projects Agency, and Radiation Incorporated. Edited by the Technical Staff, Research Division, Radiation Incorporated. Pp. x+298. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

THIS volume is the record of the papers presented at the Symposium on Space Trajectories held in December 1959 and attended by representatives of various companies and institutions in the United States. The items vary widely in their quality and length. R. Hermann contributes a lengthy and excellent paper discussing re-entry trajectories and the problems of hypersonic flow. There is an admirable review of space manœuvres and rendezvous by A. R. Tanguay, and D. C. Hock supplements this with a useful survey of optimization problems, based largely on D. F. Lawden's work. Several papers deal with the detailed computations of satellite orbits, covering both the choice of analytical method and the numerical techniques: the phase of rampant individualism which at present characterizes this complex subject is reflected by the papers, in which, rather bewilderingly, almost every author advocates a different method. It will probably be some years before natural selection weeds out the weaker theories and methods. Perhaps the weakest method (despite its author's commendations) is that described on p. 266, in which the apogee height obtained, approximately 1,400 miles, is apparently in error by about 1,000 miles.

Volumes of individual papers are often marred by errors, inconsistencies and repetitions, and this book has its share. The corporate wisdom of the editors (who sign themselves at the end of the preface as "Radiation Incorporated") has not prevented frequent mis-spellings of authors' names—Sir Harold Jeffreys enjoys the distinction of having his name mis-spelt in two different ways—and there are many other irritating peccadilloes. For example, the legend on Fig. 2 on p. 217 is irrelevant and should have been removed; but Fig. 2 then becomes the same as Fig. 3 on p. 83 and is superfluous. Also the introductory pep-talk, which has the curious title "Military Space: Taking the High Ground", should have been pruned of its nationalistic sentiments before being included in a technical book. To sum up, the book contains much to interest the specialist, but cannot be recommended as a general introduction to the subject.

D. G. King-Hele

RUMINANT DIGESTION AND NUTRITION

Digestive Physiology and Nutrition of the Ruminant

Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, 1960. Edited by Dr. D. Lewis. Pp. ix+297. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 50*s*.

THE importance of ruminants in agricultural economy has provided an effective stimulus to the study of their nutrition for more than a century. Perhaps it was natural that the earlier work was concentrated rather on dietetics, with obvious practical applications, than on the physiological and biochemical mechanisms governing the digestion and metabolism of feedstuffs. Nevertheless, it was recognized, long ago, that the most striking difference between ruminants and the familiar monogastric animals was the capacity of the former to utilize cellulose as an energy source comparable in value with starches and sugars. It is one of the most remarkable features in evolution that only very rarely have animals acquired the capacity to secrete cellulolytic enzymes—and this capacity does not seem to occur in any vertebrate. Cellulolysis in the digestive tract is essentially a function of microbial activity and, in the ruminant, provision has been made for this activity to take place in the so-called fore-stomach—a complex of three pouches developed from the cardiac region of the more primitive stomach. This development has led to extraordinarily complicated anatomical, physiological, microbiological and nutritional consequences. For more than a century, sporadic—and often brilliant—research contributions have been made in several facets of this field, but it was not until the 1930's that the subject started to receive the attention it merited; since then interest has steadily accelerated. So rapid has been the development that a student must needs go to the original literature and to a handful of reviews for a survey of the field.

Especially welcome therefore is this recent publication. It is a collection of 23 short papers presented at the University of Nottingham's Seventh Easter School in Agricultural Science, held in 1960. In the space of some 270 pages it was obviously not possible to give a complete account of the present status of this subject, but a great deal of ground is covered by the 25 authors, all of whom have made notable research contributions. Each chapter is complete

with a short list of selected references which will give ready access to the appropriate literature.

The work is conveniently divided into three sections. Under "Physiology of the Rumen" consideration is given to the movements and nervous control of the stomach, rumination, eructation, absorption and the flow of digesta. It is interesting to note that the phenomena associated with rumination, in spite of intensive, imaginative and ingenious research, still presents many unsolved problems. So too does the complex sequence involved in eructation—this latter function is especially important as its failure leads swiftly to the fatal consequence of hoven or bloat, a major disease in both dairy and beef cattle.

In the section "Metabolism in the Rumen" a brief outline of rumen microbiology is given, accounts of the fate of carbohydrates, proteins and lipids, and discussions of special techniques applicable to these fields. The microbiota of the rumen must surely be one of the most fascinating collections of organisms in a natural habitat ; the delicately poised system in the rumen, so easy of access for research, is potentially a most fertile source of material for the ecologist who is interested in the dynamics of natural microbial populations.

The third section, on "Ruminant Nutrition", makes no attempt to cover the conventional fields which are adequately treated in many text-books. Attention is focused on a few actively growing points, notably endocrine relations in metabolism and nutrition, and the synthesis of vitamins and microbial proteins in the rumen ; also, the recent advances in ruminant physiology have awakened renewed interest in energy utilization, to which two chapters are devoted.

In spite of the limitations inseparable from any compilation based on a series of conference papers, this book will prove valuable to all students of ruminant nutrition and will provide a good background for readers interested in the metabolic diseases of ruminants.

I. W. McDonald

TUMOUR METABOLISM

The Glycolysis and Respiration of Tumours

By Alan C. Aisenberg. Pp. xiii + 224. (New York: Academic Press, Inc.; London : Academic Press, Inc. (London), Ltd., 1961.) 8 dollars.

THE present time seems quite appropriate for a dispassionate survey of our present-day knowledge and theorizing on the respiration and glycolysis of tumours. This subject begins with the original observations of Otto Warburg, made in 1923. The technical basis of these was important in itself: the introduction of the use of the thin slice of fresh tissue as experimental material and the development by Warburg of manometric methods based on those of Haldane and Barcroft; two of the most fruitful contributions to experimental biochemistry.

These are accepted. What is controversial concerns mainly the interpretation of these measurements in relation to the problem of cancer, and on this subject argument has tended to be hasty, intemperate and acrimonious, especially by the 'all-or-nothing' school, who will accept only a single key to cancer, and will discard everything less universal. On the other side of the picture, Warburg himself and his more fervent disciples have sometimes appeared to push their case too far against the opposing weight of evidence, or at least to turn a blind eye to inconvenient experimental findings.

The primary beliefs of the Warburg school are briefly as follows: (*a*) in relation to tumour growth, and to the neoplastic process in general, energy-yielding metabolism is all-important; the rest is secondary. (*b*) While growing tissues, both embryonic and neoplastic, when maintained anaerobically have a high rate of glycolysis (that is, lactic acid formation from glucose), aerobically only the tumour tissues show strong glycolysis. (*c*) There is a disturbance in the respiration of tumours which is not only the cause of their aerobic glycolysis but also which is fundamental to the nature of cancer.

As Dr. Aisenberg clearly brings out in his very satisfactory review of the relevant factual information, it is quite easy to assemble data either in support of each of the above dogmas, or contrary to them. Indeed, of the 538 literature references cited, a fair proportion are regarded by their authors as providing decisive evidence one way or the other. The fact that honest and capable workers should find themselves so divided, even sometimes on the experimental findings themselves, is a good reason for the publication of this book, which presents an unbiased and reliable account of the work up to 1960 on the respiration and glycolysis of tumour tissue, and, where the comparison is available, on related normal tissues.

In spite of all the work summarized in this useful book, I am left with an opinion not essentially different from that which I put forward in *The Cancer Review* in 1931: "Perhaps it might even have been assumed to be improbable from the outset that such fine distinctions as exist between various types of malignant and non-malignant growths should be wholly explicable in terms of simple measurement of respiration and glycolysis". Surely the respiration of a tissue should not be assessed for this purpose solely in terms of its rate of oxygen uptake, when it is now very much clearer than when the above words were written that tissue respiration is merely the overall expression of an exceedingly complex chain of enzymic reactions, involving alternative substrates, alternative metabolic pathways, alternative co-enzymes, and alternative routes of energy utilization. Nor can the entity 'aerobic glycolysis' be conveniently divorced from the complex balance of enzymic activities and sub-cellular structure by means of which it is regulated. Finally, the control of synthetic reactions, especially of biosynthesis of protein and of nucleic acids, needs to be considered in relationship to what Krebs has called 'pacemaker' reactions, which, though all-important in regulation of cell metabolism, may constitute only a small part —though a determining one—in the overall crude picture of 'respiration and glycolysis'. The author of this carefully 'neutral' book might not perhaps be willing to go so far, but his well-balanced record of thirty-seven years of experiment, inference and controversy should assist towards a happy issue to this vexed question.

The book is generally excellently produced by the Academic Press, and is a publication of the Cancer Commission of Harvard University. Minor blemishes occur in the spelling of some names (Lipmann, p. 67; Stickland, p. 217); the date of Warburg's book, *Stoffwechsel der Tumoren* (p. 218), was 1926; "anaerobic glycolysis", attributed on p. 14, line 8, only to the very young embryo, should read "aerobic glycolysis".

F. Dickens

XUM

A Clinical Prospect of the Cancer Problem

By Prof. D. W. Smithers. (Monographs on Neoplastic Various Sites.) Pp. xv+232. (Edinburgh and London: E. and S. Livingstone, Ltd., 1960.) 37*s.* 6*d.* net.

MORE than five years ago Prof. D. W. Smithers undertook to act as general editor of a series of monographs on neoplastic diseases at various sites, and four books in this series have already been published, dealing with cancer of the lung, bladder, rectum and œsophagus. Other volumes dealing with the prostate, brain, larynx and pharynx are also in preparation. The justification for this piece-meal way of approach to the cancer problem is derived from the fact that, from the clinical point of view, cancer of each organ of the body has its own particular characteristics, affecting ætiology, pathology and treatment.

Each volume in the series of monographs on neoplastic disease at various sites is edited by someone with special experience in this particular field, and this series of books is proving to be of exceptional value to those doctors who are engaged in the diagnosis and treatment of cancer of the different organs of the body selected for inclusion in the series. But to fill in the gaps another monograph was also obviously needed, one which would survey the cancer problem as a whole yet still preserve the clinical point of view. This necessary introductory volume has now been written by Prof. D. W. Smithers himself.

He has unique qualifications for this task. Not only is he the general editor of the whole series (and incidentally the originator of the idea) but also his work as director of the Radiotherapy Department of the Royal Marsden Hospital and Institute of Cancer Research has brought him into contact with clinicians and research worers concerned with every aspect of human cancer.

Prof. Smithers begins by saying that his book presents "a personal view of the cancer problem". It tends to be "speculative and philosophic" in character. It is certainly original, stimulating and provocative, and provides an admirable introduction to this useful series of monographs.

Cosmology

By Prof. H. Bondi. Second edition. (Cambridge Monographs on Physics.) Pp. viii+182. (Cambridge: At the University Press, 1960.) 30*s.* net.

THE call for a new edition of this book is a tribute to the excellence of the book itself and also an indication of the vitality of its subject. Ever since the 'modern' work in cosmology was started by Einstein in 1917, or thereabouts, as soon as it has seemed to some workers that a stage had been reached when little more could be done, other workers have revitalized the subject by some major idea or discovery. Thus, for example, Prof. Bondi and his colleagues produced the ideas of continual creation and of steady-state cosmology in 1948. Walter Baade discovered the need for a far-reaching revision of the distance scale in 1952, and this year Prof. M. Ryle and his colleagues have published their new surveys of remote radio sources. To some of us it seems indeed that the subject still awaits the key discovery that may be expected to show the significance of all that has been done hitherto. Anyhow, anyone wanting to make a serious study of the subject at present, and probably for a long time to come, needs to know all that is contained in this book, and he is fortunate indeed in being given the material in such a clear, vigorous and stimulating form.

W. H. McCrea

The Use of Organic Reagents in Inorganic Analysis

By A. I. Busev and N. G. Polianskii. Translated from the Russian by J. T. Greaves. Pp. vi+76. (London and New York: Pergamon Press, 1960.) 21*s.* net.

THIS translation from the original Russian has been produced, for reasons of celerity and economy, by non-letterpress setting and photolithography. The final result, although lacking the attractiveness of conventional printing, gives a quite legible text.

No booklet of seventy pages could possibly present a detailed practical description of the many applications of organic reagents in the analytical field. The present volume provides extended abstracts of the more important papers on the use of organic reagents, these abstracts being supported by a comprehensive bibliographical and reference section.

The first chapter of the book is devoted to general problems associated with the use of organic reagents, for example, specificity, functional groups, masking reagents, effect on redox potentials, and on catalytic reactions, etc.

The determination of cationic elements is dealt with sequentially according to the group system of the Periodic Classification, the presentation being both lucid and informative. No mention is made of anion-reagent complexes.

This is a useful reference book for the practising analyst or researcher in analytical methods, and it also gives an interesting indication of the keen interest taken by Russian chemists in the work on the application of the newer organic reagents to quantitative analytical problems.

D. T. Lewis

Die Cactaceae

Handbuch der Kakteenkunde. Von Curt Backeberg. Band 5: Cereoideae (Boreocactinae). Pp. xxxix-xliv+2631–3544+tafeln 212–255. (Jena: Gustav Fischer Verlag, 1961.) 107 D.M.

HERR BACKEBERG'S fifth volume concludes his vast systematic treatment of the Cactaceae with the remaining groups of the Cereoideae. A final supplementary volume with indexes is announced for next year. The present one is bigger than its predecessors because of the account of the important genus *Mamillaria*, which occupies more than 400 pages. The author recognizes 301 species and then adds descriptions of more than 100 "Wenig bekannte Arten", in alphabetical sequence, in accordance with the similar list in R. T. Craig's Handbook (1945). Herr Backeberg's adoption of the spelling *Mamillaria* rather than the original *Mammillaria*, to be regarded as an orthographic error, will be hotly contested by many botanists, who will read his discussion in the lengthy footnote on p. 3091. Lewis and Short's Latin Dictionary certainly quotes *mammilla* as an incorrect form of *mamilla*, the diminutive of *mamma*. The International Code of Botanical Nomenclature, however, retains the spelling *Mammillaria* in its list of *Nomina Generica Conservanda*.

N. Y. Sandwith

SCIENCE AND THE CLASSICS*

By Prof. B. FARRINGTON
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THE circumstances of our age seem to present us with a very real problem which may fittingly be discussed under the title of "Science and the Classics". In response to an international challenge we are engaged in expanding our educational services. The urgent need is for more scientists, more technologists, more engineers. This means a relative, if not an absolute, decline in the study of the humanities. Is this shift in the character of our education good or bad? Is there anything in the claim that the study of the humanities produced a more balanced type of man? Is there in the scientific mode of training any tendency against which it is desirable to be on our guard?

I think that there is a tendency among scientists to suppose that the physical world alone is real, and that the very successful methods they have devised for dealing with it are applicable to every aspect of life. This, to my way of thinking, is a dangerous illusion. I am one with Schweitzer in believing that "all progress in discovery and invention evolves at last to a fatal result, unless we keep control over it by a corresponding progress in our spirituality"[1]. I am convinced, with him, that "in the last resort, the practical can be realised only through the ethical". I fear that a preponderance of scientific elements in our education, and a decline in humanistic studies, may render us incapable of exercising effective control over our science of Nature.

Science, though it has its roots far in the past, is in the present acceptation of the term a recent growth. Four hundred years will cover its history; and the speed of its recent development gives it a still more modern aspect. But four hundred years of science have not put four thousand years of civilization out of date. There is a provincialism in time as well as in space. To think that everything has been changed in the past four hundred years is a mistake. The world did not begin with Galileo and Newton. All culture is not merely scientific culture; but all culture is a mode of thought, and man should try to think with the whole of his past. This is the specific business of the humanistic disciplines.

But I trust that no one will take me for an enemy of science. Twenty-five years ago I concluded a little book on the history of Greek science[2] with some words I should like to recall. "It seems impossible", I then wrote, "that a stable society should ever establish itself without the positive knowledge science has to offer as a universal subject of education and the morality science imposes as an element in its law." I still stand by that to-day. But I did not believe then, and I do not believe now, that either the aims, or the method, or the achievement of science give grounds for hope that a stable society could ever be founded on science alone. The sciences are the creation of a specialized use of the mind. They are not its only valid use. It is not true, as seems now to be believed by many, that it is science alone which brings us into contact with 'reality'. The most important aspects of reality for man are those that concern his own actions and aspirations, and these, for the most part, are not amenable to quantitative analysis; they elude the scrutiny of the exact sciences. Thought about them has not been radically altered by the discovery of the scientific method. Conceptual thought is an older and more necessary tool than the scientific method. Understanding is not always helped by the accumulation of information. Poverty of ideas is a more effectual bar to wise action than the absence of statistics. The intuitive powers of the mind, dependent more on sympathy and imagination than on exact information, open the door to the moral and æsthetic world, that is to say, to that aspect of reality ultimately decisive for our happiness. Neither technological nor sociological knowledge is of any use to us, as Shelley observed long ago, if we lack "the creative faculty to imagine what we know, and the generous impulse to act what we imagine".

Francis Bacon was of the same opinion. He never countenanced the illusion that scientific or technological progress in themselves could benefit mankind. They could only bring an increase of power, which could be used for good or evil according to the disposition of the will. His opinion is particularly worthy of attention since his advocacy played a decisive part in the technological revolution. He convinced his age both of the need for an immensely increased store of factual information, and of the dazzling potentialities of the science that could be raised on this foundation. But we should not pass over his fear lest "from the unlocking of the paths of sense and the enkindling of a greater light in nature any unbelief or darkness should arise in our minds to shut out the knowledge of the divine mysteries". Nor did he leave us in any doubt as to what was, in his eyes, the chief of the divine mysteries. "I would address", he writes, "one general admonition to all, that they consider what are the true ends of knowledge and that they seek it not either for pleasure of the mind, or for contention, or for superiority over others, or for profit, or fame, or power, or for any of these inferior things; but for the benefit and use of life; and that they perfect and govern it in charity." These essentials of which Bacon speaks, namely, "the true ends of knowledge", "the benefit and use of life", and the supreme duty of charity, are no part of a scientific training. The question to consider is whether the apprehension of these aspects of reality has, in fact, gained anything by the scientific revolution and whether it stands to gain anything in the future.

Bronowski, in his recently published *Science and Human Values*—a wise book, well-suited to our present needs—forcefully argues the case for a fundamental identity between the moral, æsthetic, and scientific imagination. The processes, he claims, by which we deepen and advance our understanding of the good, the beautiful, and the true, are essentially one. In each case men seek to find unity in variety, to connect things which previously lay apart. The creative flash in which the unity is perceived is in each case the same. I am sure there is some truth in this contention. Particularly do I appreciate his insistence that science is not the collecting of facts, but the creating of concepts. With an argument conducted at this level I have no quarrel. Nevertheless,

* Substance of a lecture delivered at Hatfield Technical College on June 2.

I suspect that at the humdrum level of ordinary daily practice there remains an urgent problem which Bronowski's analysis ignores.

Sir Bernard Lovell recently said that, of all the scientists that have ever existed on Earth, 90 per cent are alive at the present moment. Think of the sudden expansion of scientific training that these figures imply. Tens of thousands, hundreds of thousands, of young men and women are now undergoing an initiation into scientific method. I welcome this. All my conscious life I have wished to see it. I believe, and hope, that it is destined to increase. But I am not convinced that such a transformation of human consciousness as this shift in education implies is unattended by the gravest threat. We have the prophetic fear of Bacon, we have the humble testimony of Darwin, that the necessary drudgery of science may dim the more imaginative powers of the mind on which lively apprehension of moral and æsthetic reality depends.

That this is so, I feel. Why it should be so is an obscure question on which I must now try, not very optimistically, to shed a little light. First, I should like to direct attention to the vast difference in time-scale between moral and æsthetic development on one hand and science on the other. No anthropologist that I have ever heard of claims to find among what we call primitive peoples the equivalent of a modern scientist. But anthropologists do profess to recognize in these simple societies philosophers, sages, leaders, law-givers, creators of admirable and viable patterns of social life. Prof. Ashley Montagu, for example, asserts that "in spite of our enormous technological advances we are spiritually, and as human beings, not the equals of the average Australian aboriginal or the average Eskimo—we are very definitely their inferiors"[2]. The reason for this early development of morality seems plain. If any human society at all is to exist, and such have existed for hundreds of thousands of years, its members must evolve or accept some rules of living together, some sense of community, some morality. This is true even of food-gatherers. Morality can be highly developed in technologically primitive societies.

The exploitation of the external environment, however, which is rudimentary science, has always introduced problems of social organization which swamp moral standards. Schweitzer's dictum that "all progress in discovery and invention evolves at last to a fatal result, unless we can manage to keep control over it by a corresponding progress in our spirituality", does not apply only to modern conditions, but also to all history. It was a commonplace with the philosophers of classical antiquity. Neither Platonist, nor Epicurean, nor Stoic would be surprised to find that the period of our greatest triumphs in science and technology coincides with the overwhelming of our humanity and sense of community. The gas chamber, the napalm bomb, the systematic employment of every refinement of torture are characteristics of a high material civilization. The bond of human fellowship is very old and very fragile. Morality has usually come off second best in its encounters with the social problems created by the increase of prosperity. The same appears to be true of man's æsthetic sensibility. It is an early starter and an easy victim. As the cave-paintings of primitive men remind us, hunters could create works of art revealing a power of visual imagination which eludes potters and farmers.

I have said that we should try to think with the whole of our past. One of the thoughts most strongly induced by this practice concerns the unevenness of progress. The most striking gains prove, on examination, to have involved serious loss. An example is alphabetic writing. This surely, one would think, is a wholly beneficent achievement of human ingenuity. It was, in fact, a necessary pre-condition of the emergence of large-scale organization, yet it wrought so decisive a change in human consciousness that it all but obliterated the memory, and certainly destroyed the understanding, of the age-old culture of pre-literate societies. The invention of the alphabet blinded men to the knowledge of some thousands of years of our human story. Much research and a great effort of the historical imagination were needed to restore to the twentieth century an appreciation of the oral cultures which writing destroyed. Yet so accomplished a stylist as Plato was near enough to the world of oral tradition to view the dissemination of the alphabet with a critical eye. He had two main fears. One, that writing would impair the memory. Has it not done so? "Without writing," notes Tom Harrisson, "memory is perfect, tradition exact." Secondly, Plato felt that a book was an inadequate substitute for a living teacher. We may note that the two greatest teachers in the European tradition, Socrates and Jesus, never wrote a book. In our own educational practice at its best, books are treated only as an adjunct to the spoken word.

But this is far from being the end of the matter. There are other penalties literacy exacts : sluggish powers of observation, inability to learn directly from observation, helplessness in the face of Nature. We learn to read and forget how to look and listen. Paul Radin speaks of "the distortion in our whole psychic life, and in our whole apperception of external realities, produced by the alphabet". Schweitzer reminds those charged with the education of simpler peoples that "civilization began with the handicrafts and agriculture, not with reading and writing". Our own Boy Scouts, Girl Guides, and other such organizations usefully make up the deficiencies of an education too exclusively concentrated on books.

Furthermore, we should note, as particularly relevant to our subject, that the unique vitality and charm of ancient Greek literature arises from the fact that it remained for long much closer to the oral tradition than our modern manner of studying it suggests. Greek boys, like ours, studied grammar; but grammar then had a much fuller meaning than it has now. It meant, not so much learning to read, as to read aloud. A written text was then treated like a musical score. The grammar lesson included elocution. It taught the correct delivery for the various styles: epic, lyric, tragedy, comedy. It concerned itself also with pitch and intonation, on which the beauty of composition was thought to depend, and on strict observance of phrasing without which the full meaning was obscured. So long as Greek civilization lasted, in every city they founded round the Mediterranean, in the Black Sea, or the Near East, they preserved their cultural tradition by setting up schools where the boys learned to *recite* Homer, and theatres where adults *heard* great tragedies performed. As for silent reading, it can only be said that the Greeks did not have a word for it. So when Philip in the *Acts* encountered the Ethiopian eunuch he *heard* him reading the prophet Esaias. Some five centuries later when the servants of St. Ambrose *saw* him reading the scripture

and *heard* nothing, they took it for a miracle. Silent reading had begun. Literature was on the way to becoming voiceless, a matter of visual symbols which the eye is drilled to take in as quickly as possible. Mercifully, in this case, we manage to some extent to retain the advantages of both worlds. Mere information we take in quickly by the eye alone. Something of the glory of the spoken word as it existed in classical antiquity is preserved in the Church, in the theatre, and in our new medium of mass communication, broadcasting.

These are examples, chosen almost at random, to remind us of the ambivalent nature of social change. Technological advance sets society problems too difficult for its moral resources to handle. The acquisition of powers of abstraction weakens the visual imagination. The art of writing, which in one sense may be said to constitute the memory of mankind, in another sense destroys that memory by externalizing it. Why, then, should we wonder if it should appear that the development of the exact sciences damage the apprehension of all those aspects of reality that are not amenable to its method of analysis.

That this, in fact, is so, the history of ancient Greece strongly suggests. The Greeks were not yet a scientific people when, between the eighth and fourth centuries B.C., they created that literature which still to-day constitutes the most vital part of what we Europeans generally mean by the humanities. Before the end of the fourth century, epic, lyric, drama, history, and philosophy already existed in their most original and perfect examples. Nor is it true, as seems now sometimes to be supposed, that this literature was a sort of superfluous adornment of life, inessential, but adding grace and charm. This is by no means so. On the contrary, it was creative in the fullest sense of the word. It was a new mode of thought, an exploration of reality, and, indeed, the very substance of a new reality arising out of the old. Men then thought about themselves as they had learned how to do from Homer, Pindar, Aeschylus, Thucydides, and Plato. The minds, the characters, the passions of men, the destinies of individuals and of States had been portrayed by thinkers profoundly capable of reflexion on their national traditions and their personal experience, and gifted with the imagination to embody them in new art forms: and the intellectual adventure was carried farther still. Men were equipped with the concepts and the vocabulary to discuss intelligently the facts of society and politics. But not only that. They succeeded also in distinguishing between life itself and its representation in the various arts. They created not only a philosophy of life but also a philosophy of art. Aristotle, for example, freed the criticism of the drama from its entanglement with morals and politics, assigned it a distinct function and analysed its constituent elements in terms which are still the stock-in-trade of all critics. His pupil Aristoxenus did a similar service for music, distinguishing acoustics, which is properly concerned only with the physical antecedents of sound, from music itself in which the sounds are considered in their relation to one another in an integrated composition. Thus was created the humanist consciousness, a new awareness of social life through the instrumentality of literature and art. This, to my way of thinking, is still fundamental, and in no way replaceable by later modes of thought.

Then, after the fourth century, a change in the mental climate set in. Greek geometers set to work on the observational material accumulated by the Mesopotamian star-gazers, and the exact science of astronomy was born. It was discovered how to plot accurately the paths of the Sun, Moon, planets, and stars against a spherical background of which Earth was the centre. Thus was inaugurated a space-probe of a breath-taking kind, even if as yet it was accomplished only in the imagination of the mathematician. The new science had practical advantages for agriculture, navigation, and geodesy. It also adapted the religious consciousness to the new political conditions. Improved communications had begun to unify mankind. It was no longer expedient that different peoples should be divided by their local religious allegiances. The mathematical heavens became the chief object of religious awe. The countless anthropomorphic divinities of sky, sea and land were one by one identified with Sun, Moon, planets, and stars. All peoples were united in a common adoration of universal gods.

So far, so good. But it was not long before this stupendous astronomical conception took on an astrological complexion. When the Sun, who rules the day, and the Moon, who rules the night, had been identified with Apollo and Diana, that is with gods in human shape, when Venus, the goddess of love, and Mars, the god of war, when father Jupiter, strong in wisdom, and Mercury, guide of travellers and friend of thieves and business-men, had been installed as bright luminaries in the sky, was it to be supposed that this lessened their influence over the affairs of men? Their influence, now that they were enthroned in the heavens, was stronger than ever. What is more, if only the correct technique could be devised, it was calculable. This technique the astronomers bit by bit perfected. It is a mistake to think of astrology as a popular superstition. It was a highly sophisticated way of misinterpreting the universe. Long before astrology was invented the people had their humbler ways of guessing what the future had in store. It was the astronomers, bending their mathematical brains to the task, who established the astrological technique. So vast, so complicated, so daring, so seductive a conception was the work of superior minds. The creators and defenders of this crippling superstition were the Hipparchuses and the Ptolemies. They had, in the language of Bronowski, brought together in one creative flash of insight two aspects of reality that had previously lain apart and fused them into one. It was to take something stronger than science to tear them apart again.[4]

Here, then, is a subject supremely worth pondering. The astrological view of the universe presented, in a degree unapproached by any other ancient system, the aspect of an exact science. Yet despite its scientific aspect and the fascination exercised by the exactness of its procedures, it represented a decline in culture, an impoverishment of personality, an enslavement of the will, a diminishment of man's moral and spiritual nature. Man had previously been understood in terms of what the poets, historians and philosophers had made of him—a being not exempt from weakness and villainy, but yet versatile, adventurous, resourceful, enterprising, adaptable, aspiring, and in great measure his own master. Now he had shrunk to being the puppet of the stars, whose relation to the powers that ruled his destiny could be represented in a mathematical diagram. No longer a wily much-enduring Ulysses, a magnanimous Hector, a Prometheus hurling defiance at the gods, a wise law-giver, a subtle politician, or a

martyred philosopher, he had become a helpless lay-figure spread-eagled in a series of concentric circles in which revolved for ever those celestial gods whose influence, streaming down into every limb and organ of his body, determined both his physical and mental constitution and the pattern of his days.

It is often imagined that this harmful superstition was dissipated by the dawn of scientific knowledge and scientific thought. This reading of history is scarcely true. Its great strength was the scientific armature which supported its fantasies, and its opponents were humanists of one sort or another. Epicurus, for example, in antiquity, whose name is, indeed, inseparably associated with the atomism of Democritus, but as the one who asserted in the teeth of that deterministic creed the autonomy of the will. It was in the name of that same freedom of the will that St. Augustine fought astrology, drawing, I believe for the first time, a clear distinction between it and the genuine science of astronomy. At the Renaissance again it was essentially this question of freedom, presenting itself this time in the guise of the dignity of man, that brought a Pico della Mirandola into the ranks of the opponents of astrology. It is in this spirit too that Shakespeare makes his Cassius exclaim :

> "The fault, dear Brutus, is not in our stars
> But in ourselves that we are underlings."

Men had awakened from a sleep of some two thousand years in which they had allowed themselves to be imposed upon by the exaggerated claims of what was, in fact, a genuine advance in knowledge. Their awakening took the form of the resumption of those modes of thought which, though lacking the exactness of the new geometrical astronomy, were yet better adapted to handling those aspects of reality on which man's happiness ultimately depends. Astrology had given man confidence to believe that his understanding could reach out to embrace the stars ; but it was the stars that leaned down to hold him in their embrace. Astrology made man bold enough to declare himself a citizen of the cosmos ; but the tyrannical cosmos retorted that man was no citizen but a slave. It was only when the older humanistic culture had again asserted itself that man could take a more sober view of himself and resume his character of a humble lover of wisdom, neither boasting of his citizenship nor trembling as a slave.

This ancient example of the divergence between humanism and the exact sciences would not be worth dwelling upon were it not that it has something typical about it. Consider, for example, as a parallel from the modern world the history of Cartesianism. The contribution of Descartes to science (I can speak of it only at second-hand) was very great. He welded together Greek geometry and modern algebra, thus making a powerful instrument for the exact analysis of physical phenomena. But he was deficient both in æsthetic sensibility and historical sense, so that no warning voice held him back when it occurred to him to apply his mechanistic philosophy to problems of life and society. The results were disastrous. Regarding as but elegant trifling all that the senses, the imagination, and the memory had to offer, he trusted to the pure intellect alone and regarded the world of the analytic geometer as the sole reality. He arrived by this path at two major conclusions. The first was that the only existence he could be absolutely certain of (except as the result of a tedious argument) was his own. The second was that animals were only machines. He was devoted to vivisection and explained the cries of his tortured dogs as the crash of broken machinery.

From the first of these opinions, namely his solipsism, his friend Gassendi, who as a Christian defender of Epicureanism was doubly steeped in the humanist tradition, tried in vain to wean him. "You will say", he writes in a letter to Descartes, "'I am mind alone' . . . But let us talk in earnest, and tell me frankly, Do you not derive the very sounds you utter in so saying from the society in which you have lived ? And, since the sounds you utter are derived from intercourse with other men, are not the meanings of these sounds derived from the same source ?" But Descartes could not take the point. Small wonder that Thomas Hobbes, who also knew him, was wont to say that "had Descartes kept himself wholly to geometry he had been the best geometer in the world, but that his head did not lie for philosophy".

As for the second of his pronouncements, that animals are machines, it is as good an example as one could wish of that reductionism which has been called the occupational disease of scientists. Descartes, because he had succeeded in arriving at exact knowledge of certain mechanical principles, was convinced that these principles must be equally valid in a wholly different sphere. For tens of thousands of years man had been in close relationship with the other animals, and his knowledge and understanding of them had not failed to profit thereby. It is certain that without his alliance with the dog man could never have held his own against the larger Carnivora. The dog was the fifth column through which man breached and conquered the animal kingdom. Similarly the ox and the ass, the camel and the elephant, helped him to exploit his physical environment, rendering essential service to agriculture and to trade. Where would the hunter and the shepherd have been without his dog, the trader without his pack-animal, the jungle-clearer without his elephant ?

Through an experience lasting over many thousands of years man had gained an insight into the character and mentality, as well as the physical capacities, of animals both wild and domestic. He appreciated both their resemblances to himself and their essential differences. The knowledge lacked quantitative precision, but it was both true and tested. What was more, it was knowledge of whole animals in their normal habitat. All this accumulation of wisdom was ignored by Descartes' unhistorical definition. Basing himself on his laboratory experience he brushed aside all the animal lore of the past with the curt remark that "the greatest of all the prejudices we have retained from our infancy is that of believing that the beasts think". But, long before Descartes and his experiments in vivisection, men had been capable of distinguishing between the mentality of man and that of animals without losing the capacity to distinguish between animals and machines. Man had been defined by the Greeks as the rational animal *par excellence*, but to animals the Greek thinkers had allowed sense-perception and memory, and even a limited capacity for organizing experience. Now all this traditional folk-wisdom, together with all these careful philosophical distinctions, is cast aside by a man labouring under the delusion that the only way in which animal behaviour can be understood is to treat it as if it were a branch of mechanics. This is what I meant when I gave the caution at the outset of this

article that we should not allow ourselves to be dazzled by our most recent achievements but should try to think with the whole of our past. The tradition of French thought which Descartes was brushing aside can be studied equally in the humanist Montaigne, the Huguenot Palissy, or the Catholic Richeome[5].

These remarks are not intended to belittle either the greatness or the humanity of Descartes. He was, as his biographer William Wallace says, "a type of that self-reliant, harsh, and abstract spirit of science to which erudition and all the heritage of the past seems but elegant and unworthy trifling". But he had also caught from Francis Bacon a warm faith in the humanitarian spirit of science. "I believed", he remarks with regard to his discoveries in natural philosophy, "that I could not keep them concealed without greatly sinning against the law which obliges us to procure, as much as lieth in us, the general good of all men. For they have shown me that it is possible to arrive at knowledge which is very useful to life, and that instead of the speculative philosophy which is taught in the schools, a practical philosophy may be found. By means of this, knowing the power and the action of fire, water, air, stars, heavens, and all the other bodies which environ us, as distinctly as we know the various trades and crafts of our artisans, we might in the same way be able to put them to all the uses to which they are proper, and thus make ourselves, as it were, masters and possessors of nature." No, Descartes did not lack humanity; but this only makes the more significant that typical kind of scientific blindness which seeks the explanation of the phenomena of consciousness in terms of the physical sciences.

This blindness is not a phenomenon of the seventeenth century but is as alive and vigorous now as ever it was. A recent controversy in *The Observer* shows us Dr. N. S. Sutherland of the Institute of Experimental Psychology, Oxford, insisting that "the behaviour of animals and men can ultimately be explained in terms of the chemistry of the nervous system"; and that it is arrogant to suggest that "because we have not yet found a full explanation in physical terms for some piece of behaviour, there cannot be one". Now behaviour is a big term. It is not covered by what can be observed in a laboratory under laboratory conditions. To a humanist human behaviour covers the whole field of human history from the appearance of man on Earth to the present day. For Vico, the great eighteenth-century opponent of Cartesianism, reality was not covered by physical reality. It included all man's customs and laws, all his forms of association and his institutions, all his arts and crafts, all literature and the fine arts, all politics and religion. In this field also knowledge is a possibility. But its methods, procedures, and modes of explanation are different from those of the natural sciences, and, perhaps, more difficult. To imagine that chemistry and physics can throw light on them is to make the mistake the old Greeks made before Aristoxenus explained to them that music was not the same as acoustics. It is to suppose that a musical instrument is real and music itself a subsidary and dependent phenomenon which will clear itself up without more ado once the physics of the instrument have been explored. It would be naïve indeed to suppose that with the intensification of scientific training on the scale now reached this sort of blindness will not spread. It is idle to hope that if we give young people the training of Descartes they will automatically be capable of thinking like Vico.

In my view it is impossible to be too deeply concerned about this problem. Within the span of my active life as a university teacher the balance tipped from humanistic to scientific and technological studies. There were sound reasons for the change, and it has been accompanied by solid advantages, by no means only on the material side of life. But the process has gone too far. A university senate used to be a majority of humanists with a sprinkling of scientists, now it is a body of scientists and technologists with a sprinkling of humanists. This change of character in the academic body has meant the emergence in university life of an absolute incomprehension of the older humanistic disciplines. They are felt to be an expendable luxury. I remember a heart-to-heart talk with a Fellow of the Royal Society in a position of great responsibility. He wanted to know why it was not possible to make a definitive translation of all the Greek and Roman classics and thus be done once for all with troublesome business of learning these dead languages. This man could understand the world of Descartes, and his distinction in this kind of knowledge had won high recognition. Of Vico's world he did not even know enough to know that he knew nothing. But we liked one another, and he was kind enough to put his helpful suggestion to me, politely, in the form of a question. However, it left me to chew sadly over a passage in one of Schweitzer's books. "To-day," he writes, "thought gets no help from science . . . The newest scientific knowledge may be allied with an entirely unreflecting view of the universe. . . Our age has discovered how to divorce knowledge from thought, with the result that we have, indeed, a science that is free, but hardly any science left which reflects."

A supreme illustration of the kind of science which divorces knowledge from thought, of a science which is indeed free but has lost the power to reflect, is supplied by the Kinsey Reports. In them an unprecedented effort has been made to bring to the masses a strictly scientific, morally neutral, factual survey of sex. Nor do I deny that these reports exemplify one of the virtues of science, absolute loyalty to the ascertained fact. I believe further that its statistics may in the long run contribute to some good end. But for Kinsey and his team sex is a purely physiological fact taken in its most material aspect because this alone is measurable. Science to be science must be exact. Sex, therefore, in these reports is divested of any specifically human quality. So understood, its analysis will present little difficulty to the experimental psychologists. The chemistry of the nervous system will come into its own and there should be little difficulty in achieving a full explanation in physical terms of this piece of behaviour. All that will be missing is the relevance of this analysis to man. It will set him a high standard of the sexual activity that may be achieved in the course of a life. But it will not answer any questions even about pleasure, still less about happiness, or love, or parenthood, or the family, or any other institution of human society; for these things do not lend themselves to exact measurement and statistical treatment. To handle such matters as these the Cartesian conception of science must be supplemented by that of Vico[6].

I come back then, to my main point, namely, the insufficiency of the natural sciences as a key to the understanding of society. It is not difficult to see why this should be so. True, there is a sense in which man is an animal like any other, a product of biological evolution: and this fact inclines us to suppose that his

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behaviour should therefore be intelligible in purely biological terms. But this is not so. When man developed speech and conceptual thinking he cut the umbilical cord which bound him to mother Nature. He then ceased to inherit his specifically human behaviour genetically and began to hand on the tradition of culture by education. In this sense man is not simply a child of Nature. He is a kind of super-Nature. His animal inheritance is only a part of him. The other part of him is his cultural inheritance, which is the means whereby he decides what use to make of his animal nature. In this sense, a limited but very real sense, man is both free and creative. If we seek to explain him purely biologically we rob him of his freedom and his creativity. If we wish to rouse and instruct in him his capacity for free creativity we can only do this by increasing his understanding of what, in fact, man has accomplished in the brief span of human history. There is no key to this knowledge in its full force and significance other than the humanistic disciplines. For the humanist the pageant of history is as much part of reality as the non-human reality investigated by the natural sciences. For the future prospects of mankind it has the tremendous value that it gives him the sense of what man has done and therefore the courage to try to do better. Man in the biological sense is the end-product of a chain of accidents, the passive outcome of a process which he may come to understand but did not in fact control. But his human history is his own creation. In it he can study his mistakes and achievements. It is an infinitely difficult but infinitely rewarding pursuit, and anything that can be done to encourage it is not wasting time and money on frills but getting fully to grips with reality. Nor is there any other means by which man can learn to take his destiny into his hands.

The pitfalls, however, are endless, and there is no lack of false prophets to deceive the elect. Who, for example, would suspect that a biologist so cultivated in the widest sense as Bergson was should nevertheless lead us up the garden path? But that is just what he did when he took his philosophy of the *élan vital* out of the biological field, in which it had originated, and used it in an attempt to explain some of the most fundamental problems of cultural history. In his *Creative Evolution* the philosophy of the *élan vital*, whether we accept it or not, was a useful hypothesis in the sense that it held together, without violently distorting their true nature, a great chain of biological observations. But Bergson did not stop there. Seduced by the success of his biological theory, he sought to unlock the problems of religion and morals with the same key. He imagined, absolutely gratuitously and without a particle of supporting evidence, that the conception of the *élan vital* was identical with the vision of the great mystics, and that this vision was the true source of progress in morals and religion. The identification is completely without historical foundation. But Bergson, though he could adopt a hare-brained notion, could not write other than brilliantly. The result is that his *Two Sources of Religion and Morality*, having already misled one generation of readers, has now, after a quarter of a century, emerged as a paper-back to mislead a still wider circle. It has, of course, the usual disabling effect of all attempts to explain the human in biological terms. For the *élan vital* is not under human control, and there is absolutely nothing for any follower of Bergson to do in the way of deepening the spirituality of the race except to wait for the unpredictable moment when the *élan vital* will inspire some mystic to utter a new oracle. But if we are ever to build Jerusalem we need some handier weapon which the average man can employ in what must be a co-operative enterprise[7].

By way of summing up let me add only this. I have not forgotten the general title of the series to which I have been privileged to contribute. We were to discuss the "Common Ground shared by Technology, Science, and Art". Neither do I forget that it is a Technical College that has inaugurated the discussion and invited me to take part in it. This emboldens me to hope that in what I have attempted to do I shall not be misunderstood. I am very much of the opinion, expounded by Sir Eric Ashby in one of his books[8], that it is a function of technology to humanize science. For it is in its technological applications that science is forced to come out of the laboratory and consider the impact of its discoveries on the everyday lives of ordinary men and women. In such a liberal atmosphere I have ventured to interpret the words 'common ground' in my own way. I have hoped to show that it ought to be common ground between us that, in spite of the overwhelming need for science and technology in the world to-day, the humanities have a distinct function. I believe that there is a fundamental aspect of reality, not susceptible of analysis by scientific methods, the understanding of which is the proper business of the humanistic disciplines.

To this sphere of reality, the sphere of human history, the approach is not by way of mathematics and the experimental method. The path is through languages and the study of the masterpieces of art and literature. These disciplines should, in my opinion, even now and in spite of other claims be given vastly increased support. This support should, of course, include facilities for the study of living languages, the knowledge of which is essential in our contracting world. But it must also concern itself with the past, through the study of what are called, perhaps prematurely, the dead languages. For they may be dead, but they will not lie down. For those of us who are heirs of the Western European tradition Hebrew, Greek, and Latin obviously come first. But we must provide also for the languages of the older civilizations of Egypt and Mesopotamia, and for the languages of other continents and civilizations. It has long been recognized as the prerogative of man that he looks before and after. We may, I suggest, be certain that our forward vision will clear when we take the trouble to understand a bit more of our past.

[1] This and other quotations from Schweitzer are from his *Philosophy of Civilization*, Parts 1 and 2 (A. C. Black, 1923 and 1946).

[2] *Science in Antiquity* (Home University Library, Oxford University Press).

[3] For this quotation and that which soon follows from Paul Radin, see Coomaraswamy, A. K., *The Bugbear of Literacy* (Dobson, 1947), where there is much supporting material.

[4] For the Hellenistic origin of personal astrology see Neugebauer, O., *The Exact Sciences in Antiquity*, 163 (Oxford University Press, 1951). A recent work, Hadas, Moses, *Humanism* (Allen and Unwin, 1961), brilliantly illustrates the character of Greek culture before it succumbed to astrology. Cumont, Franz, *Astrology and Religion among the Greeks and Romans* (Dover Publications) is the classic analysis of the victory of astrology, a process oversimplified in my brief account.

[5] For Montaigne see the end of the essay on *Cruelty*; for Palissy various reflexions on animal intelligence quoted in Morley, Henry, *Palissy the Potter*, 2 (1855); for Richeome, H. Brémond's *Hist. Lit. du Sentiment religieux en France*, 1.

[6] Lionel Trilling's essay in *The Liberal Imagination* (Mercury Books, 1961) on the *Kinsey Report* has influenced me.

[7] Bergson's unintentional distortion of the historical reality is examined by Loisy, *Y A-t-il deux Sources de la Religion et de la Morale?*

[8] Ashby, Sir Eric, *Technology and the Academics* (Macmillan and Co. Ltd.).

TRADITION, SCIENCE AND PRACTICE IN DIETETICS

THE third International Congress of Dietetics, organized by the British Dietetic Association and an International Committee of Dietitians, chaired by Miss D. F. Hollingsworth, of the Ministry of Agriculture, Fisheries and Food, was held at Church House, Westminster, London, during July 10–14, under the honorary presidency of Lord Boyd Orr. There were 916 registered members of the Congress from more than forty countries. The purposes of the programme were to place applied nutrition—or dietetics—in a historical setting and to show how dietitians are working throughout the world towards the better nourishment of all people. The programme was designed to allow time for discussion of scientific and of practical problems and for professional visits.

After the formal opening of the Congress by the Mayor of Westminster, Dr. Norman C. Wright, deputy director-general of the Food and Agriculture Organization of the United Nations, spoke on "International Collaboration in the Solution of Problems of Human Diet". He said that primitive man, in common with other animals, learned to procure food from his environment which provided the ingredients needed to ensure survival of the human race, but that with the development of a more complex civilization "this knowledge, acquired by trial and error rather than by scientific experiment, has been increasingly neglected". He traced the growth of international intervention in the problem of inadequate food supplies for the world's rapidly growing population, emphasizing "the vital role of women in ensuring the adequacy of family diets and of the urgent need to improve the status and education of women in . . . [the] less developed countries".

Mr. F. Le Gros Clark (London), in speaking of the "History of Human Diet", underlined the fact that "*Homo sapiens* is the only species whose dietary habits have had any history at all". He postulated that our primitive forebears developed preferences for a thoroughly mixed diet, of foods consumed mainly in their natural state, suited to the primitive mode of life and environment. He emphasized that under primitive conditions there would have been no need to consider the application of nutrition and no place for the dietitian. In his view, "the science of applied nutrition, and with it, the role of the dietitian, are the by-products of a 'civilized' mode of life" and that our task now is "to persuade men and women, whose elementary tastes and food inclinations were built into the species many centuries ago, to match their food habits to a completely changed mode of living".

Speakers from many parts of the world illustrated ways in which traditional food habits have proved to be scientifically sound: the most striking example was, perhaps, that provided by the traditional method in Central America of making tortillas by first soaking maize in lime water and thus making available bound nicotinic acid, a practice which doubtless explains the absence of pellagra in those countries. Many examples were also given of the dangers which follow the introduction of 'civilized' diets to primitive communities: for example, the increase in dental caries among the village children in Fiji and other South Pacific Islands which has followed the introduction to the diet of refined cereals and sugar. More than one speaker with public health experience in developing countries emphasized the prestige value in primitive communities of 'Western' foods and, in many places, the consequent neglect of locally grown foods, which are often of relatively greater nutritional value.

Then followed three and a half days of sessions for discussion and original communications on a variety of topics, including atherosclerosis, diet and ischæmic heart disease, and mother and child, with special reference to protein malnutrition. Other subjects discussed were dietetics and its history, therapeutic diets, feeding older people, survey work, teaching nutrition to nurses, school meals, catering problems and the education and occupations of dietitians.

The two sessions on various aspects of coronary heart disease provided ample opportunity for interchange of opinion and fact. Prof. J. J. Groen and Prof. G. Verdonk and their colleagues in the Netherlands and Belgium presented the results of their work on the influence of nutrition and ways of life on blood cholesterol and the prevalence of atherosclerosis and coronary heart disease among Trappist and Benedictine monks. They showed that the average blood cholesterol was higher among 168 Benedictine monks who lived on a 'Western' diet than among 180 Trappists who were vegetarian. There was more obesity among the Trappists than among the Benedictines, but no difference in prevalence of hypertension, myocardial infarcts, angina pectoris or electrocardiographic signs of ischæmic heart disease. Myocardial infarction appeared to be rarer among both Orders, but the other conditions mentioned occurred to much the same extent as in the general population of the Netherlands and Belgium. The conclusion reached was that the acute occlusion of a major coronary artery, leading to a macroscopic myocardial infarction, seemed to be related not to nutrition and low blood cholesterol, but to psychological factors in the Western ways of life against which monks were protected and that the nature of these factors required further investigation. Hypertension and diffuse ischæmic heart diseases were apparently not prevented by a frugal diet or by a monastic life.

Dr. J. Trémolières (Paris) discussed the many factors concerned in the study of diet and atherosclerosis. He emphasized the importance of obtaining more precise information on the diet, way of life and the cardiovascular condition of individuals. Prof. J. N. Morris and his colleagues from the Medical Research Council Social Medicine Research Unit presented the results of their attempts to devise a method of large-scale individual dietary surveys on middle-aged men, and Prof. G. Verdonk (Ghent) stressed the complexities of lipid metabolism and suggested that 15–20 different biochemical estimations are necessary to study the course of atherosclerosis. He also advanced the clinical opinion that to lower a patient's blood cholesterol-level is to increase his feeling of well-being. Dr. Trémolières concluded that myocardial infarction is more serious among the obese, the anxious, the smokers and those who appear to over-eat for their height and weight, and that a preventive regimen appears to be "une bonne hygiène de vie", without excessive anxiety, and a moderate diet maintaining the individual at slightly reduced weight. He suggested that not more than 35 per cent of the dietary calories should be

derived from fats and that the diet should be "sans carence en acid gras essentiels, sans huiles de mais peroxydées et . . . sans trop de frîtures". Dr. V. Socolovsky (Moscow) concluded his contribution on the same subject with the advice that one should "eat less and so live longer to eat more in the end". Prof. J. Yudkin (London), in opening the session on diet and cardiac ischæmia, emphasized the uncertainty of present knowledge. The only conclusions to be reached at the end were that more careful observation and painstaking research are required to solve this most complex problem and that, in the meantime, the only possible prescription is moderation of both life and diet.

The present state of our ignorance was also emphasized by Prof. B. S. Platt (London) when he introduced the session on the nutrition of the mother and her child. He then described recent progress in the experimental production of protein malnutrition in animals and new concepts on protein–calorie deficiency diseases, including marasmic kwashiorkor. Miss Margaret Cameron (New Zealand) described recent team work in Prof. Platt's unit to determine the protein values of foods, meals and mixtures as they are eaten by man. The protein value of a diet is affected by the quality of the protein, its concentration, the presence of certain other nutrients in the diet, for example, vitamin B_{12} or magnesium, and the energy value of the diet in relation to the energy needs of the person eating it. It is also affected by the physiological and pathological states of the individual; for example, it changes during growth or lactation and in some illnesses. She described the method of allowing for these factors in the calculation of the protein requirements of a growing child or a pregnant or lactating woman and illustrated ways in which the protein value of the customary diets may be calculated, and where necessary increased, by supplementing or substitution or by reduction of the amount of oil or sugar consumed. She emphasized the care needed to devise acceptable meals, in keeping with the tastes, religious beliefs and cultural patterns of the children and lactating mothers for whom they are intended.

At the final plenary session of the Congress, Mademoiselle Yvonne Serville (Paris) spoke of the means, indirect and direct, of conveying the findings of research on the science of nutrition to the consumer, emphasizing particularly the complexity of the science of nutrition and the need for some training in nutrition for all those concerned with food from production to consumption, and for teachers, particularly of home management and natural sciences.

This was a notable Congress with an admirable combination of discussions on science and practice, which itself was an illustration of the part dietitians can play in the interpretation of scientific findings in the interests of the consumer.

The Proceedings of the third International Congress of Dietetics, under the title *Tradition, Science and Practice in Dietetics*, have been published by Newman Books, Ltd. Copies may be obtained from the British Dietetic Association, 251 Brompton Road, London, S.W.3, or from Newman Books, Ltd., 68 Welbeck Street, London, W.1 (price £3 3*s*., postage paid).

D. F. Hollingsworth

BARIUM-140 IN NEW ZEALAND RAINWATER

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Since the testing of nuclear weapons began, attempts have been made to use the resultant fission products as meteorological tracers. A major difficulty has been to know from which series of nuclear tests the radioactivity of any sample has come. One method which has been used to age fission products, and thus to determine in which series of tests they were formed, has been to measure the rate of decay of the gross beta or gamma activity of the sample, and to compare it with observed rates of decay of fission-product samples of known age. In recent years more precise information about sample ages has come from measuring ratios of specific isotopes such as barium-140/strontium-90 and cerium-144/strontium-90, and comparing these ratios with fission ratios for these isotopes formed in different types of fission reactions. An increase in the ratio of shorter- to longer-lived isotopes is indicative of fission products of more recent origin.

In 1958 we decided to try to obtain information relevant to the problem of global air circulation, particularly with respect to the exchange of Northern and Southern Hemisphere stratospheric air, by measuring isotope ratios in rain collected at Lower Hutt, New Zealand (41° S.). In November 1958 we began measuring 28-year strontium-90 and 12·8-day barium-140 in fortnightly accumulated rain samples, and by June 1959 we were determining these isotopes in each rainfall.

Some of the results in this programme have been recorded[1]. In Table 1 are shown the only positive barium-140 depositions obtained up to June 1961. The methods used are being reported elsewhere[2]. The strontium-90 and barium-140 depositions were calculated from the measured activities of their daughter products, 64-hr. yttrium-90 and 40-hr. lanthanum-140. The measured counts, using a low background beta counter with initially type GM-4 counters and later flow-counters, were never greater than 1 c.p.m. and although repeat lanthanum-140 determinations and barium-140 plus lanthanum-140 determinations were carried out, the activities were too small to identify accurately the presence of barium-140 by half-life determinations. It is considered that the samples quoted in Table 1 did contain barium-140. The errors quoted are due to the 90 per cent confidence-level of statistics of counting only.

Reference to measurements reported in ref. 1 will show, in addition to the positive barium-140 values recorded in Table 1, nine samples out of fifty-eight measured in 1959 which showed activities slightly greater than the statistical counting error of measurement. We now believe that these are not real barium-140 results. The first five during January–July were obtained at an early stage of the programme before the routine measurement techniques were completed, and it now appears this apparent activity was

Table 1. Positive Measurements of Barium-140 in New Zealand Rainwater, Lower Hutt, 41° S.: November 1958–June 1961

Sample No.	Dates when collected from to	Rain (in.)	Strontium-90 μc./square mile	Barium-140 μc./square mile at mid-collection	Barium-140 / Strontium-90	Probable source of barium-140	Per cent strontium-90 due to French test
	1958 (2 samples) November 7–December 4						
	1959 (58 samples) January 15–December 1						
	1960 (24 samples) February 13–May 31					1960	
B 044	February 13–February 19	0·2	28 ± 1	2·5 ± 1·2	0·1 ± 0·05	February 13	0·005
B 069	March 9–March 12	1·53	57 ± 1	5 ± 3	0·1 ± 0·06	February 13	0·02
B 072	March 12–March 16	0·23	17 ± 1	5 ± 1·5	0·3 ± 0·1	February 13	0·07
B 085	March 25–March 26	0·25	20 ± 1	3 ± 1·5	0·15 ± 0·1	February 13	0·06
B 122	May 1–May 3	0·13	19 ± 2	11 ± 4	0·6 ± 0·2	April 1	0·12
	1961 (13 samples) January 7–June 1						
C 024	January 24–January 31	0·08	21 ± 1	2·4 ± 1·3	0·12 ± 0·06	December 23	0·03

probably yttrium-90 not lanthanum-140. The other four apparent activities in September/October 1959 were due to the use of an incorrect reagent blank.

During early 1959 the accuracy of our barium-140 determinations was about ± 100 μc./square mile, but this was reduced to about 1–2 μc./square mile by 1961. From the limits of detection of barium-140, the measured values of strontium-90 deposited, and a value of 1480/1 for the barium-140/strontium-90 ratio[3] for the fast neutron fission of uranium-238, the maximum percentage deposition of strontium-90 due to the October 1958 test programme of the Soviet Union can be calculated. These values are compared in Table 2 with the maximum percentage strontium-90 deposited due to this series calculated from measurements of total beta activity which will be published shortly. The percentage contributions quoted here are maximum values; it has not been demonstrated that any strontium-90 is in fact due to this series. Table 2 shows that the percentage contribution of strontium-90 from the October 1958 series of the Soviet Union detectable by our methods increases as the barium-140 decays until after about March 1959 it was no longer possible to detect an influx of debris from this source. The limits of detection for this debris based on total beta activity measurements assumed all the activity came from the series of tests in the Soviet Union. However, age estimates based on rates of decay of the total beta activity of monthly rain samples suggest an influx of fresh debris from about March 1959 at least until our measurements ceased in June 1959. Although this fresher debris could originate in the Soviet Union, it is not possible to rule out entirely the last tests in the Pacific area. Results for total beta activity suggest that the October series of tests in the Soviet Union did not contribute more than 4 per cent of the total strontium-90 deposited at Lower Hutt from November 1958 until June 1959, assuming fast neutron fission of uranium-238.

Table 2. Maximum Percentage Contribution to Fall-out in New Zealand of the October 1958 Series of Tests in the Soviet Union

Sample collection	Calculated from	
	Barium-140/strontium-90	Total β/strontium-90
1958		
November	0·2	0·3
December	3	13
1959		
January	8	3
February	32	8
March	80	1·6
April		10
May		3
June		12

During 1960–61 six positive barium-140 results were found which we believe to be reliable. These results were obtained in the six-week period after the French test on February 13, 1960, and also approximately four weeks after each of the French tests on April 1 and December 23, 1960. If we assume that the fission/yield activity ratio of barium-140/strontium-90 for the fast fission of plutonium-239 is 1820/1 (calculated from ref. 3) then the French contribution to the measured strontium-90 deposited can be found. This is shown in Table 1 for individual rains and has never been found greater than 0·12 per cent. It seems reasonable that this amount of strontium-90 could have arrived at Lower Hutt through the troposphere from the Sahara tests.

[1] *U.S. Atomic Energy Commission HASL Reports*: **77**, 34 (1960); **84**, 62 (1960); **88**, 80 (1960); **95**, 86 (1960); **105**, 90 (1961).

[2] McNaughton, G. S., and Woodward, R. N., *New Zealand J. Sci.* (in the press).

[3] Katcoff, S., *Nucleonics*, **16** (4), 78 (1958).

OBITUARY

Prof. E. H. Neville

Eric Harold Neville, who died on August 21 aged seventy-two, was educated at William Ellis School where he came under the influence of Percy Nunn, who clearly stimulated in him an innate feeling for mathematics. In 1907 he went up as a senior scholar to Trinity College, Cambridge. It had then been announced that publication of the Mathematical Tripos lists in order of merit would cease with the lists for 1909; this challenged Neville to sit the examination a year earlier than normal, and he was placed second wrangler in 1909. Two years later he won the Smith's Prize and was elected into a fellowship at Trinity, which he held until 1917.

In 1919 he was appointed to the chair of mathematics in University College (now the University) of Reading, which he occupied until his retirement in 1954. He married Alice Farnfield in 1913, who pre-

deceased him in 1956 ; they had one son who died in infancy.

At the beginning of 1914, while giving a course of lectures in India, he was charged by G. H. Hardy with the task of persuading the Indian mathematician Ramanujan to come to Cambridge. The account of this mission which he gave nearly thirty years later (*Nature*, **149**, 292; 1942) is witness to the satisfaction he derived from the successful completion of this task.

His earliest work was in geometry. In *Prolegomena to Analytical Geometry* and *Multilinear Functions of Direction* he was clearing the ground for a treatment of the whole field of differential geometry based on secure logical foundations. Unfortunately, the breadth of his interests led him to undertake other tasks and the work was never completed.

The need to organize the teaching of undergraduates drew his attention to the unsatisfactory foundations of much basic mathematical instruction and he threw himself enthusiastically into the work of the Mathematical Association. With Percy Nunn he was largely responsible for the Association's first *Report on the Teaching of Geometry*. He became librarian of the Association in 1923 and in the course of thirty years he transformed the library from a small collection which could be accommodated in his own house to the valuable reference library for teachers now housed with the University of Leicester. He was president of the Mathematical Association in 1934. He was interested in the work of the British Association, principally in connexion with the Tables Committee, of which he was chairman during 1931–47. He compiled the *Table of the Farey Series of Order 1025* and the *Rectangular to Polar Conversion Tables*. His prefaces to these works contain much that is of interest to anyone contemplating the compilation or printing of mathematical tables. He was a member of the Council of the British Association and, in 1950, president of Section A. He also served on the Council and as vice-president of the London Mathematical Society.

Out of his concern for the improvement of the teaching of pure mathematics arose a number of notes on a wide variety of topics, characterized by freshness of point of view, elegance and economy of presentation. A more ambitious work directed towards the same end was his book on *Jacobian Elliptic Functions*, designed to restore the original beauty and symmetry to a field that had tended to become somewhat unwieldy.

Neville was a man of considerable moral and physical courage—he once rescued a child from the flooded Cam in midwinter. He was essentially a liberal humanist. His love and knowledge of literature matched his interest in the history of mathematics. He was a devoted bibliophile, and it is characteristic that his private library contained not only early editions of all the works of Lewis Carroll and H. G. Wells, but also what is believed to be the most complete set of works on elliptic functions.

R. Rado

NEWS and VIEWS

Textile Engineering at Leeds : Prof. P. Grosberg

Although the wool-textile industry is so highly mechanized, graduates in engineering have so far taken little interest in the construction and use of textile machinery, and have largely neglected the opportunities that all sections of the industry provide. There is here a serious threat to the future of the wool-textile industry of the United Kingdom, and the machinery-users showed commendable foresight in endowing a research chair of textile engineering at Leeds some twelve years ago. The first occupant of the chair, Prof. A. H. Nissan, left to take up an appointment in the United States, and he is now to be succeeded by Dr. P. Grosberg, who brings to the post qualifications in pure science, engineering and textile technology. Dr. Grosberg was born in Cape Town in 1925. After graduation in physics and mathematics, and in chemical engineering at the University of the Witwatersrand, he carried out research on thin film lubrication for the degrees of M.Sc.(Mech.Eng.) and Ph.D.(Eng.). On being appointed to the staff of the newly formed South African Wool Textile Research Institute in 1950, he was seconded to the Department of Textile Industries of the University of Leeds for a two-year course of advanced training in textile technology. After a period of some four years research on worsted drawing and spinning processes in South Africa, Dr. Grosberg returned to Leeds as an Imperial Chemical Industries Fellow in textile engineering, and he was appointed lecturer in textile engineering in 1956. His subsequent investigations, in which he has had the assistance of a number of Wool Textile Research Council Fellows, have already secured for him a reputation in such widely different fields as worsted drawing and spinning, warp-knitting, and drying processes. He is now to have the stimulating task of placing the subject of textile engineering on a sound foundation in the University, and of providing the textile industry with the engineers of which it stands in such great need.

The National Bureau of Standards : Dr. H. C. Allen

Dr. Harry C. Allen, jun., has been appointed chief of the Analytical and Inorganic Chemistry Division at the National Bureau of Standards, U.S. Department of Commerce, Washington, D.C. He will direct a broad programme aimed at developing methods for measuring the chemical properties, composition, and behaviour of substances; preparing standard reference materials; and making accurate measurements and data collections on chemical systems. Since 1954, Dr. Allen has been working at the Bureau on the analysis of high-resolution infrared spectra of gases, and the theory of molecular spectra. He spent the year 1959–60 in the Department of Theoretical Chemistry, University of Cambridge. Before becoming a staff member of the Bureau, Dr. Allen was an assistant professor of physics in Michigan State College. He has also been a consultant to the Bureau.

World Population Trends

The 1960 edition of the *Demographic Yearbook* of the United Nations Organization has as its special feature an analysis of the evolution of the population of various countries, and includes a table showing

the populations of each political area in every year since 1920 (Pp. xi+621. New York: United Nations; London: H.M.S.O., 1961). The first chapter entitled "How Well Do We Know the Present Size and Trend of the World's Population?" gives a valuable account of the methods used in preparing population statistics by the United Nations Organization. It is perhaps not always realized that approximately one-half of the world's population is now concentrated in four countries: China, India, the U.S.S.R. and the United States. The Organization assesses the accuracy of the 1953 census for China and suggests that the number of inhabitants of mainland China in that year was between 553 and 612 millions, and in mid-1959 between 605 and 697 millions. This is the population about which there is the maximum degree of uncertainty, India's population is known within 11 millions, and that of the two others within 3 millions. As regards the other areas of the world, it is believed that in most estimates there is a tendency to underestimate rather than overestimate populations, though some errors will no doubt be self-compensating between different territories. Their population is estimated as being between 1,431 and 1,476 millions; the four large areas have populations between 1,416 and 1,508 millions. The world's population in 1959 is believed to be between 2,853 and 2,964 millions, so it is evident that the uncertainty about China is the major factor in the range. The demographers of the United Nations Organization estimate that the annual rate of growth may lie between 1·5 and 2 per cent per annum, resulting in a total annual increase of 44–55 million persons. Tables in the *Yearbook* include data about the economically active and the illiterate population of different countries, as well as vital statistics dealing with births, marriages and deaths. The rudimentary data on expectation of life in African countries indicate the tremendous scope for mortality reduction that still exists in that continent.

Population Statistics in the United Kingdom

THE Registrar General's Statistical Return for the first quarter of 1961 records nearly 204,000 live births, the highest figure for the first (or any) quarter for more than ten years (No. 449. Pp. 28. London: H.M.S.O., 1961. 2*s*. 6*d*. net). Marriages, on the other hand, are fewer at 84,000, compared with 89,000 a year ago. There were 177,000 deaths, giving a relatively high crude death-rate of 15·7, but infant mortality continues to decline, and at 22·9 per thousand births is an all-time low for a first quarter. It will be interesting to see whether the declining trend in the number of marriages will continue, and be followed by a decline in births. The return contains, as is usual in the first quarter, estimates of the home population of administrative counties, boroughs and urban districts at June 30, 1960, together with vital statistics for these areas. In 1960 there were only 22 deaths from poliomyelitis: the lowest number since records began to be kept.

Technical Reports of the U.S. Armed Forces

SINCE the Second World War, the Office of Technical Services had been the agent in the Federal Government for the collection, reproduction and sale of reports of Government-sponsored research to firms and organizations not performing contract work for the Department of Defense. The Armed Services Technical Information Agency is the central agency of the Department of Defense, the mission of which is to provide for the efficient interchange of military research and development reports among Department of Defense agencies and their contractors. The number of technical reports of research and development sponsored by the Army, Navy, and Air Force which are made available to the scientific and industrial public is expected to double next year through an extension of the co-operative activities of the Office and the Agency. With the growing volume of Government-sponsored research, the extension of the co-operative work between the Office and the Agency will enable the Office to offer to the public in the next fiscal year 15,000–20,000 technical reports, prepared under military auspices. The Armed Services Technical Information Agency acquires and catalogues almost 30,000 military technical reports each year—about two-thirds of them unclassified. Under the new co-operative programme, the Agency will provide copies of the unclassified reports to the Office, together with catalogue information. A further use of work already carried out by the Agency will be made in the production of the Office's periodical *U.S. Government Research Reports*, which is used as an announcement medium to inform civilian scientists, engineers, and business men of new reports which are available. The unclassified portion of the Agency's *Technical Abstract Bulletin* which, so far, has been produced only for activities directly concerned with military research and development, will become a section of the *U.S. Government Research Reports*, which, from July 1961, will be published twice a month instead of once a month as in the past. Details of the new programme are outlined in the June–July issue of *Scientific Information Notes* (3, No. 3; June–July, 1961).

The National Lending Library

A RECENT issue of *N.L.L. News*, a news-sheet of the National Lending Library for Science and Technology, indicates broadly the types of literature now available from Boston Spa (No. 1; August 1961. Pp. 4. Boston Spa: National Lending Library for Science and Technology, Department of Scientific and Industrial Research, 1961). These include translations of Russian literature, including 'cover-to-cover' translations; Russian books; the 'PB' reports issued by the Office of Technical Services of the U.S. Department of Commerce; the published reports on German industry produced by the British Intelligence Objectives Sub-Committee, the Combined Intelligence Objectives Sub-Committee and the Field Information Agency Technical (U.S.A.); and certain frequently used periodicals in English which were on a list issued from Stanmore. Loan requests for English language serials and books in languages other than Russian should continue to be sent to the Science Museum Library. With the exceptions indicated above, the National Lending Library is not yet providing a general loan service for publications in the English language, but it is expected that in about twelve months time the man-power problems will have been solved and the transfer from the Science Museum Library completed. It is then proposed to make a full-scale survey of the National Lending Library's use, non-use, and of its failures to meet requests with the view of improving its services and promoting the greater use of scientific literature.

The Use of Scientific Literature

THE *Library Association Record* for August 1961 contains an article on "The Use of Scientific Litera-

ture", by B. C. Vickery, which is of some scientific interest. Mr. Vickery summarizes, with a useful bibliography and some account of the methods of study which have been used, the kinds of information sought by scientists, the extent and nature of their reading and the forms of document used. The survey shows that the quantitative contribution to the reading and learning of scientists made by formal bibliographical tools is equalled by the contributions of present-day scanning, cited references, and of oral recommendations. Mr. Vickery recognizes, too, the importance of oral communication, but he indicates rather than discusses the implications of such conclusions as are reached, and emphasizes the need for further research on ways of improving the present position. A second article in the same issue, by W. L. Emerson, discusses an approach to book selection in the sciences for public libraries.

The Geological Society of India

In January 1959 the Geological Society of India was founded to promote the advancement of geological study and research throughout all India and to co-operate with similar institutions in other lands. One of the aims of the new Society is the publication of a journal, and of monographs and memoirs, dealing with special problems of Indian geology; and the first two volumes from this source have now appeared (*Journal of the Geological Society of India*, Vol. 1, 1959. Pp. 166+6 plates. Bangalore: Geological Society of India, 1959. Rs. 15). This periodical embraces the whole field of geological science, the first two issues comprising 25 short papers mostly of a high standard with many of world-wide interest. In the first presidential address, Dr. D. N. Wadia deals with "The Evolution of the Desert Belt of Asia", delineating briefly, but brilliantly, the overall geological problems of the vast arid tracts which stretch from the Red Sea to Mongolia. Within the past decade the number of geologists in India has increased greatly, and the potential importance of the geological contribution to national development there is becoming widely recognized. The Geological Society of India is likely to play a major part in maintaining the long-established tradition of sound work, and its publications will be warmly welcomed in geological libraries throughout the world.

Atomic Energy-levels in Crystals

Atomic Energy Levels in Crystals, by J. L. Prather, National Bureau of Standards Monograph No. 19, which was issued on February 24, presents a theoretical study of the sharp-line absorption spectra of crystals (National Bureau of Standards. Monograph No. 19. Pp. iv+84. Washington, D.C.: Government Printing Office, 1961. 60 cents). It is largely an exhaustive collection of the results of group theory that apply to the theory of atomic energy-levels in crystals, together with a detailed development of the general theory to permit application of the results to experimental problems. The discrete energy-levels observed within certain crystals are treated as being due to perturbations of the energy-levels of the free ion by an electrostatic field arising from the crystal lattice. The analytic procedures for determining the field from the charge configuration are given, and the resulting fields are classified according to their symmetry. After a general survey of group-theoretical ideas, the applicable groups are analysed in detail, and characters appropriate for both integral and half-integral angular momenta of the free ion are tabulated. The monograph contains eighteen tables and an extensive bibliography. The review on the general subject entitled "Group Theory and Crystal Field Theory", written by Dr. C. M. Herzfeld in collaboration with Prof. P. H. Meijer, which is to be published shortly by the National Bureau of Standards, Washington, treats the subject from the more abstract point of view of modern algebra and concentrates on the fundamental aspects of the theory, and will be a complementary work to that of the monograph.

Travelling-wave Tubes

The travelling-wave tube is rapidly becoming established as an essential component in microwave links used for civil communications. It is a microwave amplifier that operates on the principle of a continuous interaction between the input signal and an electron beam as they travel together through the tube, and is particularly suitable for operation at frequencies greater than 1,000 Mc./s. It has a broad band-width and high gain. An outline of the construction, characteristics, operation and typical applications of the travelling-wave tube is given in the illustrated booklet, *Travelling Wave Tubes : Their Characteristics and Operation*, issued by the English Electric Valve Co., Ltd. (Pp. 40. Chelmsford: English Electric Valve Co., Ltd., 1961). Following a general description of the tube and its associated circuitry, explained by means of a simplified schematic diagram, the booklet discusses the various types of travelling-wave tubes now commercially available. They can be classified according to their functions into three major groups—power tubes, medium power or intermediate tubes, and low-noise tubes—and these can be subdivided, according to the type of *rf* connexion used, into either wave-guide or coaxial mounting types. All the travelling-wave tubes produced by the English Electric Valve Co., Ltd., require a longitudinal magnetic focusing field for maintaining the electron stream in a concentrated beam over the length of the tube. One section of the booklet deals with focus systems for the tubes, and another with the procedure to be followed in the installation of a tube into a circuit. In addition to the use of travelling-wave tubes as microwave amplifiers in both terminal and repeater stations in communication links, the low-noise tube is being increasingly used in the input stage of radar receivers as a pre-amplifier to the crystal mixer, and travelling-wave tubes may also be used as amplitude or phase modulators, frequency changers, stable oscillators or reflex amplifiers. The booklet is intended as an introduction only to the subject of travelling-wave amplification, and for those who want a more detailed treatment a bibliography of sixteen references is provided. The abridged data listed refer to the English Electric tubes and solenoids.

Lalor Grants and Awards for 1961

The Lalor Foundation has announced the programme of awards for 1962, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open

to all nationalities. The awards may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff with an upper age limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere, for appropriate projects in the fields specified. For these awards, the stipends will normally not exceed 1,000 dollars for a single man or a woman, 1,200 dollars for a married man working at his home institution, and 1,350 dollars for a married man with principal programme at another institution. Applications and inquiries should be sent to the Lalor Foundation, 4400 Lancaster Pike, Wilmington 5, Delaware, and applications must be received by January 15, 1962.

The Bowles Memorial Travel Scholarship

DURING 1962–63, £500 will be made available to enable a scholar to travel abroad to collect plants for introduction to British gardens, and/or to study practical horticulture in other countries. Applicants must be male, not more than twenty-eight years of age on January 1, 1962, and be holders of the National Diploma of Horticulture, or students or graduates in horticulture or botany at a British university, or past or present student gardeners at the University Botanic Garden, Cambridge, the Royal Botanic Garden, Edinburgh, the Royal Botanic Gardens, Kew, the Royal Horticultural Society's Gardens, Wisley, or other horticultural educational establishments. Applications should reach the Secretary, Royal Horticultural Society, Vincent Square, London, S.W.1, not later than November 20.

The Royal College of Science and Technology, Glasgow

AN endowment of £800 has recently been made to the Royal College of Science and Technology, Glasgow, by Mrs. Hilda M. Beilby, of Darlington, Co. Durham, daughter-in-law of Sir George Beilby (who for sixteen years was chairman of the governing body) and grand-niece of Lord Kelvin. The endowment will be known as the Kelvin Prizes, and it will be used to provide awards in the subjects of natural philosophy and applied mathematics. Thus, in conjunction with the endowment made recently by Lord Fleck to commemorate the work of Sir George Beilby, the College is now able to offer awards in mathematics, natural philosophy, metallurgy and chemical technology.

University News : London

THE following appointments have been announced: J. P. Quilliam, reader in pharmacology at St. Bartholomew's Hospital Medical College, to the chair of pharmacology tenable at that College; Dr. S. J. Taylor, lecturer in the Department of Pure Mathematics, University of Birmingham, to the University readership in mathematics tenable at Westfield College.

The title of professor of electrical engineering in the University of London has been conferred on Dr. J. H. Westcott, in respect of his post at the Imperial College of Science and Technology.

The following readerships, tenable at the Imperial College of Science and Technology, have been announced: Dr. J. R. Butler (pure geochemistry); Dr. C. B. Alcock (chemical metallurgy); Dr. J. N. Hunt (physical oceanography); Dr. J. A. Kitchener (mineral processing). Mr. J. C. G. Coleridge, lecturer in the University of Leeds, has been appointed as reader in physiology tenable at the Royal Free Hospital School of Medicine. Prof. J. N. Phillips Davies, professor of pathology at Makerere College, has been appointed as reader in morbid anatomy at the Postgraduate Medical School.

Sussex

THE following appointments have recently been made: Dr. C. Eaborn, reader in physical-organic chemistry in the University of Leicester, to be professor of chemistry; Dr. R. J. Blin-Stoyle, Fellow and lecturer in physics at Wadham College, Oxford, and senior research officer in theoretical physics in the University of Oxford, to be professor of theoretical physics; Dr. K. F. Smith, Fellow of Sidney Sussex College, Cambridge, and lecturer in physics in the University of Cambridge, to be professor of experimental physics.

Announcements

THE Department of Chemical Technology, Bradford Institute of Technology, is organizing a special short course on "Recent Developments in the Chemistry and Technology of Polymers" at the Institute during November 17–18. The fee for the course is £2 5*s*., and further information can be obtained from the Registrar, Institute of Technology, Bradford 7.

THE Institute of Physics and the Physical Society announces a symposium on "High Energy Nuclear Physics", to be held in the Physics Department of the Imperial College of Science and Technology, during March 26–27, 1962. Further details can be obtained from the Administration Assistant of the Institute and Society, 47 Belgrave Square, London, S.W.1, during December 1961.

A SYMPOSIUM on "Redundancy Techniques for Computing Systems" will be held during February 6–7, 1962, in the Department of the Interior Auditorium, *C* Street, between 18th and 19th Street, N.W., Washington, D.C. The symposium is being sponsored by the Information Systems Branch, Office of Naval Research. Further information can be obtained from Miss Josephine Leno, Code 430*A*, Office of Naval Research, Washington 25, D.C.

AN international symposium on "Enzymic Activity of the Central Nervous System" will take place in Gothenburg, Sweden, during June 18–21, 1962, under the auspices of the Neurochemical Commission of the World Federation of Neurology. Further information can be obtained from the secretaries: Dr. G. B. Ansell, Department of Experimental Psychiatry, Medical School, Birmingham 15, and Dr. A. Lowenthal, Institut Bunge, 59 Rue Philippe Williot, Berchem-Antwerp, Belgium.

ERRATUM. In the issue of *Nature* for September 16, p. 1142, it is announced that Dr. J. E. Stanworth is to succeed Mr. L. J. Davies. It was not made clear that Dr. Stanworth succeeds Mr. Davies as director of research of Associated Electrical Industries (Rugby), Ltd., Laboratories.

XUM

THE INTERNATIONAL MEASUREMENT CONFERENCE

THE second International Measurement Conference was held in the Engineering Societies Building, Budapest, during June 26–July 1. The host organization was again the Hungarian Scientific Society for Measurement and Automation, with Prof. György Striker as executive secretary.

The Conference was attended by some 550 delegates from twenty-two countries and 128 papers were presented in four, or sometimes five, simultaneous sessions. The four working languages of the Conference were English, French, German and Russian; but in fact no translation into French was available and for the first three days translation into English was practically non-existent. This breakdown in the translation service, coupled with the failure to provide reprints of the papers until the final day of the sessions, seriously reduced the value of the Conference.

The 128 papers were divided into eleven sections covering metrology and terminology; technology and organization in instrument manufacture, electronic measuring instruments, border questions related to measurement and automation, instruments and methods for measuring geometrical and mechanical quantities including geodetic measurements, instruments and methods for measuring thermal quantities, instruments and methods for physico-chemical measurements, instruments and methods for measuring electrical and magnetic quantities, instruments and measuring methods in radio engineering and instruments and methods for measuring radioactive radiation. Since papers from four different sections were presented concurrently, it was impossible for any one person with more than the most narrow field of research to attend all the papers in which he was interested.

The standard of the papers varied considerably from the specific to the most general survey and from new basic research to the re-statement of well-known principles: a reduction in number would have benefited all concerned.

Among the papers of general interest, that by T. Kemény (Hungary) described an automatic electronic potentiometer in which the conventional slide-wire had been replaced by a strain-gauge bridge. The four, all active, gauges of the bridge are attached in the standard configuration to a singly supported cantilever beam of low-energy-consuming elastic material. The free end of the beam is deflected by a cam driven through a gear train by the servo motor driving the pen carriage, until the out-of-balance voltage generated by the bridge is equal to the voltage being measured. Advantages claimed are no slide-wire maintenance or wear, high linearity and infinite resolution, and ease of generation of logarithmic and other non-linear scales merely by changing the cam. Other transducers, such as electromagnetic, capacitative and inductive, were suggested, but only the strain gauge bridge has been tried experimentally.

A method for measuring small capacities of about 100 pF., with an accuracy of 1 per cent, was presented by K. Tarnay (Hungary). The method is said to combine the advantages of both heterodyne and resonance methods. Essentially, the unknown capacity and a standard are switched, at mains frequency, alternately into an oscillatory circuit, the output being fed to a phase-sensitive rectifier and thence to a phase-sensitive voltmeter.

A paper by three Soviet authors dealt with a new instrument for the accurate measurement of power, current and voltage at frequencies up to 20 kc./s. A null method is employed in which the unknown alternating current or voltage is used to heat a resistance element, the temperature of which is monitored by a detector element consisting of a bank of some 35–40 thermocouples in series. The thermal e.m.f. is measured by means of a potentiometer and a direct-current voltage is then switched in in place of the unknown alternating current and is adjusted to give the same heating effect as measured by the potentiometer. The equivalent direct-current voltage is then measured by means of a second potentiometer of high accuracy. Good accuracy is obtained with power factors as low as 0·1 by using two heaters. The main source of error is said to be the reactance of the heater elements and shunts in the circuit, and this limits the frequency range. The overall error in comparison of alternating and direct current is less than 0·05–0·2 per cent, depending on range.

Prof. Gy. Striker (Hungary) discussed the possibilities of a long-range planning of instrumentation in a planned economy and suggested that the basic data required were the expected trend in capital investment in the respective industries and the index of instrumentation, that is, the ratio of instrument investment to capital investment and the expected changes in this index. Examples were given of how these factors may be fitted to equations to predict instrument requirements for a longer period.

An electronic statistical quality control apparatus which indicates average and variance directly was discussed by A. Ambrózi (Hungary). The method requires that the parameter to be controlled may be presented by means of a suitable transducer as a proportional alternating current. A batch of components is fed into the unit and the corresponding alternating-current signals are fed to a simple computer which then indicates the mean and the variance on meters, the chief advantage being the speed with which the required data can be obtained.

Two papers on measuring systems for flight testing of aircraft by J. K. Stotz and V. R. Boulton (United States) both stressed that main expenditure was the cost of data reduction which could take up to ¾ man-hr. per point. Strain gauge transducers are used wherever possible because of their inherent accuracy—0·1 per cent—and because they enable standardization of supply voltages and amplifiers as well as being easily calibrated by electrical simulation before and after a test. Recording is by magnetic tape either airborne or ground station, and overall system accuracies of 1–3 per cent may be obtained. Typical recorders have a band-width up to 90 kc./s. at a tape speed of 60 in./sec. Frequency multiplexing is used, up to 10 channels per tape track being common, while for low-frequency data, time division multiplexing at a rate of 900 samples/sec. is used. Such attention to the design of instrumentation and data handling equipment has reduced the data reduction cost by a factor of 4, produced 100 times as much data, which are four times more accurate, and are available for analysis in 1/15 of the time.

S. G. Rabinovitch, B. A. Soliber and V. I. Tchervyakova (U.S.S.R.) presented details of electrical measuring-instruments based on the photo-compensating amplifier. These included multi-range measuring units with a sensitivity up to 5×10^7 divisions per volt, panel amplifiers with 0·1 per cent accuracy and semi-automatic potentiometers, with an accuracy of 0·03 per cent, for instrument checking, etc.

B. Sternlicht (United States) described an indirect but extremely accurate method for measuring film thickness on bearings. He pointed out that there is a direct correlation between film thickness and temperature and he has used temperature-sensing elements for the measurement of the film thickness as well as the film temperature.

A novel technique for re-using resistance strain gauges was put forward by A. Bray (Italy). He pointed out that gauge factors cannot be given to an accuracy better than about ± 1·5 per cent, yet in many applications it is not possible to calibrate the gauge after it has been attached to the surface under test. The method entails using a second carrier of mica between the gauge and the test surface. The author used Eastman 910 cement, but it should be remembered that this is water-soluble. After the gauge has been used it may be recovered by splitting the mica. Experience has shown that the thickness of the mica must not be greater than 0·03 mm., and it must be about 6 mm. larger than the paper or foil base of the gauge. The mica will support 4,000 microstrain, and gauges have maintained a constant gauge-factor while being used and recovered four or five times.

T. M. Dauphinee presented the paper by Dauphinee, Kirby and Preston-Thomas (Canada) on the accurate measurement of temperature and depth in the ocean. Temperature was measured by means of a bifilar-wound copper-resistance thermometer cemented on to a thin-walled copper tube to give close thermal contact with the sea-water; accuracies of ± 0·01 per cent to depths of several kilometres have been achieved, as have accuracies of 0·2 per cent in depth measurement. Two methods were used for measuring depth, the sensing element of one being the bulb of a commercial mercury-in-glass regulator, change in depth being measured by the lowering of the control temperature that results from reduction in volume of the bulb due to the increase in pressure. The second method uses a water boiling-point apparatus with a thermocouple to measure the boiling temperature. An interesting planetary type of slip-ring was used to enable the cable to be wound on a drum winch. This consists of a copper stator and rotor, electrical connexion between the two being maintained by four hollow cylinders of thin spring bronze acting as planet wheels. The system was said to have thermal e.m.f.'s of less than 0·1 μV. and resistance variations of less than 0·01 ohm.

T. A. Perls (United States) described the use of pyroelectric transducers for heat-transfer measurements. A typical transducer of lead zirconate titanate 0·25 cm. in diameter and 0·05 cm. thick has a heat sensitivity of approximately 10 mV./W./cm.^{2} with a 1 M.ohm load. At present it is necessary to calibrate the transducers if accuracies to better than ± 20 per cent are required: a quartz lamp in a gold-plated reflector has proved satisfactory for this purpose, over the range 100–0·1 W./cm.^{2}. Advantages of pyro-electric transducers suggested include small size, low cost, good frequency response, high output, good linearity and ageing, and little sensitivity to temperature.

Dr. Cz. Graczyk (Poland) presented an account of a new method of measuring rate of flow of coal dust. The method is based on the application of a Venturi tube; two pressure drops are measured and it was shown that the dust concentration may be represented as a function of the ratio of the two pressure drops.

The measurement of dynamic flow by means of hot-wire anemometers of the constant-current and constant-temperature type were discussed by I. Kidron (Denmark), who pointed out the superiority of the constant-temperature type which will operate at frequencies several hundred times higher than a constant-current unit because of the reduced effect of the heat capacitance of the wire. The upper frequency limit increases with flow speed and is higher than 50 kc./s. at 100 m./sec. air speed. Kidron then discussed a newly developed hot-film transducer consisting of a thin platinum film fused to a glass support to give greater mechanical strength. The dynamic response is slowed by the heat capacitance of the glass support, but is still only 5 db. down at 50 kc./s. for 100 m./sec. air speed.

A novel bi-parametric displacement transducer with good frequency response and high sensitivity was described by Prof. N. Racoveanu (Rumania). The transducer consists of a magnetic core coil with a variable air-gap and also a condenser with variable plate-spacing. In use both coil air-gap and plate-spacing are varied together, modulating the frequency of a resonant circuit in a more linear manner than may be obtained by varying inductance or capacity alone. Designs with a plate condenser and a cylindrical condenser have been examined and a design with multiple concentric cylinders offers greatest linearity. Over the linear range of 2,000μ a sensitivity of 0·25 kc./μ is obtained in the frequency-range 370–870 kc./s.

A. Haas (Hungary), in his paper on the calibration of contact type surface thermometers, stressed the need for intimate contact between the detector and the surface under investigation and complete thermal-equilibrium between the detector, the surface and the surrounding atmosphere. The ultimate aim was 100 per cent thermal coupling, as was achieved by the immersion technique with the ordinary mercury-in-glass thermometer. He then discussed a detector consisting of a thermocouple junction set in a copper block which had been developed at his Research Institute, and which is capable of accurate results with only 60 per cent thermal coupling, and in spite of varying ambient temperature. Another surface pyrometer with an accuracy of 1 per cent was described; this consisted of two iron constantan couples separated by a layer of mica and connected in opposition across a sensitive galvanometer. A heating element above the second couple enables conduction and convection losses to be compensated. The instrument is calibrated against a third couple set into the calibrating surface. It was emphasized that during calibration it was necessary to ensure that thermal coupling should be the same as would be met in use, and thus for calibrating a surface pyrometer for use on rotating drying cylinders, a special calibrating surface was designed. This cylinder was oil heated and thermostatted, and its circumferential speed was set so that approximate thermal similarity with practical conditions may be achieved for a small area of the surface and the atmosphere surrounding the pyrometer. The cylinder

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has a Ward Leonard speed control and built-in thermocouples brought out via slip-rings so that the true calibration surface temperature is obtained.

Some ninety-five scientific institutions and manufacturing companies exhibited at the second International Instruments and Measurement Exhibition, which was held concurrently with the conference. The usefulness of the exhibition was reduced considerably by the lack of stand attendants and absence of literature on some stands. It was generally agreed by competent observers that nothing was shown by Eastern European countries which was in advance of equipment available in the West, and in many cases development in the former appeared to be 5–10 years behind the West. No Russian equipment was shown. Among Western exhibitors, emphasis was mainly on process instrumentation and control, particularly for the chemical industry, and on radiation counters of all types, while the Eastern countries exhibited more laboratory measuring equipment and in particular microwave equipment in the 8–10 cm. band for telephone and television uses.

Equipment exhibited by the Hungarian Measuring Instrument Research Institute included a six-channel transistorized amplifier for pressure pick-ups, strain gauges, thermistors, etc., with an input impedance of 10^{12} ohms and an output impedance of 1 ohm, with a frequency-range of direct current to 5 kc./s., and better than 5 per cent linearity. A transistorized servo amplifier measuring 6 × 6 × 20 cm. contained a two-stage pre-amplifier and a class *B* push–pull power amplifier stage, with an output capacity of 12 W. and working temperature range of −40–+50° C. Other transistorized modular blocks of the same dimensions included current and voltage amplifiers, 400 c./s. RC oscillators, a direct-current amplifier with a gain of 100 and a non-contacting relay which was basically an inductive pick-off from a moving pointer unit, with a sensitivity of ± 0·5 mm. pointer movement and a switching capacity of 500 m.amp. at 50 V. supply.

A self-compensating ionization vacuum gauge on show was a transistorized miniaturized version of the Modion gauge and covered the range 10^{-9}–10^{-3} mm. in a single span. A differential β-ray thickness meter suitable for measuring coating weights of paper or impregnation weights of fabric had an accuracy of 1 per cent for an impregnant weight-range of 0·5 to 2 × the fabric basis weight. The time constant of the measuring unit was variable between 5 and 20 sec. Several other examples of β- and γ-radiation measuring equipment were on display.

The Czechoslovak Export Agency (KOVO of Prague) showed a long range of electronic equipment and accessories, including a high sensitivity direct-current oscilloscope with a band-width to 7 Mc./s. 3 db. down; alternating-current sensitivity 9 mV./cm., direct-current 25 mV./cm., input impedance 1 M.ohm at 30 pF. or 70 ohms direct current, time-base 5–500 kc./s., with provision for external input to *X* plates. A two-channel oscilloscope had a sensitivity of 10 mV./cm. and band-width to 1 Mc./s. with beam switching at 120 kc./s., and a two-gun oscilloscope with similar sensitivity had a frequency-range to 2 Mc./s. 3 db. down. The O.P.D. oscilloscope for slow-speed phenomena had a 25-cm. diameter, high-resolution tube. Maximum sensitivity was 100 mV./cm., a band-width of 0–6 kc./s., and a slowest time base sweep of 1·5 min. The 'Meopta' oscilloscope camera shown had an *f*/1·8 lens of 35-mm. focal length and held 6 m. of 35-mm. film. It is a continuous recording camera with a maximum film speed of 1,000 m./sec.

A semi-automatic valve tester with punched card setting up was shown also, and an electronic null point indicator with CRO indication for indicating alternating-current bridge balance.

The Polish Exterior Trading Co. for Electrical Technology, Warsaw, showed a limited range of equipment, somewhat out of date by Western standards. Also exhibited were chemical balances and thermographs, a phase-contrast microscope and a projection microscope, refractometers and pH meters, a disappearing-filament-type optical pyrometer and a twin-beam oscilloscope with a band-width of 10 c.p.s. to 5 Mc./s. A valve test meter looked about 1945 vintage. The sole electromagnetic flow meter exhibited was on the stand of Eckandt of Stuttgart, together with a proportional pneumatic controller, diaphragm operators, a twin-channel recorder and various Bourdon tube and diaphragm pressure units, and a smart-looking range of edge-on panel meters.

Precision slide-wire potentiometers were exhibited by Carl Zeiss of Jena. These were to *DIN R*5 standard with linearity of 1 per cent and a torque movement of 35 gm. cm. for the smaller sizes. Armoured and sealed versions were also available for use in basic heavy industries and in corrosive atmospheres. A precision instrument potentiometer requiring a torque of less than 2 gm. cm., and a small, low-inertia, magnetic clutch were also shown. A photomultiplier with a cathode diameter of 140 mm. and maximum gain of 10^7 was on display together with selenium cells with a spectral response said to be nearer the human eye than any other available, making it eminently suitable for colour matching, etc.

M. A. W., Messergate and Armaturenwerk, showed a self-balancing potentiometric recorder with a 250-mm. scale-length. Sensitivity was 0·1 per cent, reproducibility 0·5 per cent, response time better than 1·5 sec., minimum range 2 mV. f.s.d. suppressed zero available, models with 1, 3, 6 and 12 channels, chart speeds 0·2–3,600 mm./hr. This appeared to be the standard recorder on Eastern European equipment. A miniature recorder of the chopper-bar type was also shown and a thermocouple temperature-indicator/transmitter/controller of the lamp and pointer-vane type with 0–5 m.amp. transmission.

The most interesting pneumatic control equipment was to be found on the stand of G.S.T. (Automatic Temperature Control C.), Berlin. As well as high- and low-pressure pneumatic differential potential cells and a force-balance electropneumatic converter for thermocouple e.m.f.'s was a well-engineered three-term controller with push-button selection of hand or automatic control and balance, with a proportional band setting between 2 and 1,000 per cent, integral and derivative action times of 0·36 sec. to 200 min. and a sensitivity of better than 0·01 per cent. Also on display was a flow-metering unit which monitored the flow-rate of the main stream fluid and at any desired time interval would meter into it by means of a solenoid valve any desired proportion of a second liquid component. Fritz Hollige (Freiburg) demonstrated the 'Helcoscriptor' high-speed recorder available with 1–6 channels. All can be used with heated stylus on sensitized paper writing and are flat to 100 c./s. for 30-mm. recording width, or with cold stylus on carbon paper flat to 130 c./s. Sensitivities of 1

mm./V. input and paper speeds up to 200 mm./sec. are available.

Hottinger Measuring Techniques, Ltd., Darmstadt, showed a range of miniature inductive pick-offs suitable for carrier frequencies of 5 and 50 kc./s., the minimum range being 20×10^{-4} mm. f.s.d., and a maximum operating temperature of 90–120° C. according to type. Strain gauges with a minimum gauge-length of 3 mm., and which would withstand a maximum strain of 2 per cent up to 200° C., were also exhibited together with a strain gauge bridge torque-meter transmitting a maximum torque of 1,000 metre kiloponds, at 4,000 r.p.m. with a reproducibility of 0·5 per cent and an output of 1·5 mV./V. supply at maximum torque loading; silver/silver graphite slip-rings with up to 12 channels, with brushes that may be replaced during running, were capable of running at 6,000 r.p.m. continuously and 12,000 r.p.m. intermittently.

Krohne of Duisburg displayed their range of rotameter-type flow-meters with magnetic pick-off of float position to provide remote indication, and a float-type density meter with inductive pick-off which fits directly into the process stream line and is independent of fluid velocity; the most sensitive range is 0·99–1·01 gm./c.c. and the accuracy of this range is ± 1½ per cent. Heating or cooling coils and built-in temperature compensation are available.

AB Lars Ljungberg, Sweden, demonstrated the 'Agnar' burette, a new and ingenious approach to precision metering, titrating and decanting. The burette has no tap, and is essentially a syringe in which the piston is positioned by means of a screw drive. Various syringe sizes are available, the 2 ml. size delivering 1 ml./rev. of the coarse knob and 12 µl./rev. of the fine knob. The drive is reversible, thus the burette is equally suitable for drawing off any volume from 1 µl. to 20 ml.

The Yokogawa Electric Works, Ltd., Tokyo, exhibited a range of direct-current precision measuring instruments including a good-looking miniature portable Wheatstone bridge. Panel meters were contemporary in style but appeared crudely manufactured and designed without regard to their function of precise indication of a measured quantity. Fully transistorized printed-circuit strip-chart recorders of about 4-in. and 8-in. chart-width had self-folding charts facilitating reading of back records. A three-term electronic controller was on view but no information about it was available.

A new instrument under development by DISA Electronik measures departures from constancy of the angular velocity of rotating equipment at speeds up to 100,000 r.p.m.

J. A. PLACE

ASSESSMENT OF PAIN IN MAN AND ANIMALS

A THREE-DAY symposium on the assessment of pain was organized by the Universities Federation for Animal Welfare (UFAW) in collaboration with Prof. C. A. Keele, presided over by Sir Russell Brain, and held in the Department of Pharmacology at the Middlesex Hospital Medical School during July 26–28. UFAW had been asked to arrange such a symposium by Dr. C. Radouco-Thomas, secretary of the Collegium Internationale Neuro-Psycho-Pharmacologicum in Geneva, who made many useful suggestions. In his presidential address, Sir Russell Brain referred to the usefulness of bringing together the peripheral and central aspects of the neurology of pain and the need for reconsidering views which at present are widely held on the subject; he said that UFAW's interest in the latter arose from the fact that pain sensations in many species appear to differ little from those experienced by man.

After Dr. K. D. Keele (London) had reviewed the history of theories of pain from primitive times, Prof. C. A. Keele (London) outlined a technique used by himself and his colleagues in which a blister is formed on the arm and the blister base is then stimulated in various ways, the resulting sensations being reported by the subject. The receptors appeared to be stratified in depth. The sensation at low levels of stimulation was not painful, and he proposed the term 'metæsthesia' for it under those conditions. Dr. Robert Smith (London) dealt with the quantitative relation between stimulus and sensation and described metæsthesia produced with potassium and polypeptide stimulations.

Prof. J. A. B. Gray (London) took the chair at a discussion of peripheral pain mechanisms. Dr. A. G. M. Weddell (Oxford) dealt with the structure of the nerve terminals in the skin as they appeared in electron and optical microscopy. Prof. Y. Zotterman (Stockholm) dealt with the specificity of cutaneous nerve fibres and the search at sub-microscopical level for the cause of this specificity. Dr. A. Iggo (Edinburgh) discussed the conduction in single *C* fibres of impulses initiated by noxious stimuli in the viscera, muscles and skin. Prof. H. Hensel (Marburg) showed a film record of afferent impulses in human single cutaneous nerve fibres. Dr. W. Koll (Göttingen) described the effects of morphine and other drugs on nociceptive spinal reflexes. An open discussion of the specificity of peripheral mechanisms followed.

Prof. D. Hill (London) presided over a session on central mechanisms of pain. Dr. D. R. Bowsher (Liverpool) and Prof. D. Albe-Fessard presented a joint paper showing that somatic sensations fall into two classes: discriminative sensations travelling by lemniscal pathways to the cortex via the thalamus and entailing a perception of location, and non-discriminative sensations travelling by extra-lemniscal pathways. Dr. G. Gordon (Oxford) described the electrophysiological approach to central mechanisms of somatic sensation. Dr. P. W. Nathan (London) dealt with traces of pain remaining in the nervous system, as in the case of phantom limbs. Dr. J. Hankinson (Newcastle upon Tyne) described the relief of pain by destruction of intracerebral structures under the guidance of stereoscopic radiography. In the ensuing discussion, Prof. J. Scherrer (Paris) and Dr. G. L. Kidd (Liverpool) suggested possible mechanisms of phantom pain, and Dr. M. R. A. Chance (Birmingham) directed attention to audiogenic seizures which do not arise in rodents unless these are deprived of escape routes.

Prof. O. L. Zangwill (Cambridge) took the chair for a session on the assessment of pain in man. Dr. H. K. Beecher (Boston, U.S.A.) discussed some working hypotheses and described his work on the effect of drugs on high-level intellectual performance.

He considered that data from experimental pain are often difficult to relate to clinical experience. Dr. J. D. Hardy (New Haven, Connecticut) described work on the pain threshold in relation to measurement of the intensity of human pain and the effects of analgesics. Dr. R. W. Houde (New York) said that an analysis of quantitative data on pathological and experimental pain in terms of the 'ridit' unit could establish a relation between these two different types of data. Dr. C. Wilson (Liverpool) discussed the factors affecting the placebo response, which may give rise to bias even in blind experiments. A general discussion of psychophysical correlation problems followed.

In a session devoted to the assessment of pain in animals, with Prof. C. A. Keele (London) in the chair, Dr. D. D. Bonnycastle (Jersey City), Dr. R. L. Kitchell (Minnesota), Dr. H. O. J. Collier (London), Dr. C. Radouco-Thomas (Geneva), Dr. K. Soehring (Hamburg), and Dr. J. Jacob (Paris) discussed the testing of analgesic drugs on animals. The final session, in which Prof. C. A. Keele again took the chair, was devoted to practical applications to animals used in research. In the unavoidable absence of Prof. A. Haddow, Dr. E. J. Delorme described several arrangements devised at the Chester Beatty Research Institute for the comfort of the animals used. Major C. W. Hume (UFAW, London) directed attention to the desirability of ensuring that experimentation on animals be restricted to scientifically qualified persons who recognize their responsibility towards the rodents and other animals used, the training of technicians and of beginners in research being particularly important in this respect. A discussion on legislation for the protection of laboratory animals followed.

A report comprising the full text of the papers with summaries of the ensuing discussions is to be published by UFAW and will, it is hoped, be available early in 1962.

SOCIAL STUDIES IN BRITAIN

IN the debate on science in the House of Commons on July 10, Mrs. Judith Hart urged the need for a social sciences research council, referring particularly to the contribution which social science could make in dealing with the special problems of the new towns, in urban research and development generally, in the National Health Service, and in the economic and social problems arising out of automation and industrial change in general. Mr. D. Freeth, Parliamentary Secretary for Science, did not deal with this subject in his reply in that debate, but it was raised afterwards by Mr. A. Albu in a debate on August 4. In the meantime, on July 18, Mr. Freeth had stated in answer to a specific question from Mrs. Hart that the Council for Scientific and Industrial Research is reviewing the support given by the Department in this field, and considering whether there is need to alter the present organization for encouraging and supporting these studies. Mr. Freeth said that the Minister for Science expects to receive the Council's views in the autumn, and on August 4 he added that the House would expect the Minister to await the Council's advice, though he was authorized to emphasize the Minister's great interest in the social sciences. The Minister would note very carefully the views expressed in the debate; Lord Hailsham believed that more work should be done in the universities and elsewhere in this field, and his continuing study would be directed to strengthening the social sciences in future.

The debate on August 4 was notable for the very full review of the whole position of the research effort in Britain in this field at present, and warm appreciation of Mr. Freeth's statement was expressed by Mr. F. Peart. Mr. Freeth began by referring to the report of the Clapham Committee in 1946, as a result of which the University Grants Committee made earmarked grants from 1947 until 1952 for the social sciences at a steadily increasing rate. A change in policy led to the discontinuance of earmarked grants after 1957, but at the present time the Department of Scientific and Industrial Research is making grants and awards totalling £140,000, the £40,000 mentioned by Mr. Albu covering grants to universities and colleges of advanced technology only. For this purpose, the Department has a special committee, and in addition the Industrial Grants Committee of the Department has made a 5-year grant to the National Institute of Industrial Psychology, depending on the amount of money raised for the purpose by the Institute. A list of investigations supported by the Human Sciences Committee of the Department is available, and although all these awards are related to industry they cover a wide field. Mr. Freeth cited as examples research at the Birmingham College of Advanced Technology into social factors affecting relations and individual performance in factories; at the University of Durham into worker–management relations in four industries in the north-east of England of differing techniques; at Brunel College, into the subjective nature of judgments made in the inspection and quality control of manufactured goods; at the University of Leicester into problems of the employment of married women in hosiery factories; and at the University of Oxford into plant-operating skills in relation to automation.

In this context Mr. Freeth referred to the dissemination of the results of such research; for example, the Ergonomics Conference held in September 1960 and the series of meetings organized to introduce the topics discussed to the research associations.

About £60,000 a year is being expended on research in this field at the Department's own laboratories, including, for example, the work at the Warren Springs Laboratory on ergonomics, such as the design and study of key-boards and a study in conjunction with the University of Manchester of secondary modern school-leavers. At the Building Research Station investigations are being made of the design and provision of flats for old people and the design of offices and hospitals. The Road Research Laboratory is continually examining the personal characteristics of road-users in relation to their behaviour on the road, and the National Physical Laboratory is investigating motor-vehicle noise, the effects of the light in a room on colour rendering, and readers for blind people, and the British Iron and Steel Research Association and the British Boot and Shoe and Allied Trades Research Association have human science and ergonomic sections.

The Medical Research Council is spending some £250,000 in this field, at its own research units and

through grants for research undertaken in the universities. At Graylingwell Hospital, Chichester, the Council has a unit with a programme including the evaluation of a community mental health service to determine the social and clinical factors which favour admission of patients to hospital and those which favour treatment at home. The unit was also studying the effects on the family of caring for a member who is mentally ill. Work in the social sciences at the universities, however, must depend largely on the importance attached to it by the universities, and the extent of their resources, but it is also influenced by the scarcity of first-degree courses in sociology as such and the difficulty in attracting postgraduate students.

Mr. Freeth referred to considerable expenditure on research into such subjects as criminology and penology by the Home Office, though he did not indicate the amount. Lists of such researches were given in a written answer by the Minister of State at the Home Office on April 13, 1961. Other Departments finance work on such subjects as perception and reaction to extreme environmental conditions, and grants are also made by certain local education authorities. How far this should be done by central bodies and how much within the Departments is a difficult problem. Mr. Freeth made no specific reference to the Ministry of Education, although in opening the debate Mr. Albu had commented on the absence of such research by that Ministry, and had referred to a memorandum recently submitted to the Minister for Science by the Parliamentary and Scientific Committee.

The memorandum pointed out that the total expenditure on empirical research on education in Great Britain was less than £125,000 a year, or about 0·014 per cent of annual expenditure on education; and it pointed out that most university research education is carried out part-time either by staff or students for higher degrees, most of whom are practising teachers with full-time jobs. There are only ten research fellowships devoted to empirical research in education, and most of the research carried out by teachers and local education authorities loses its principal value because it has no skilled advice on such matters as experimental design, test construction and statistical analysis. The memorandum urged the establishment of an educational research council with a substantial budget and an independent chairman under the direction of a committee of the Privy Council and reporting to Parliament through the Lord President of the Council.

Mr. Freeth said that the work of the Social Survey of the Central Office of Information, on which some £129,000 is expended, also covers a wide field, including inquiries on behalf of Government economists and statisticians with a special interest in economic development, inquiries into health and social problems, and inquiries into the development of techniques for supplementing and completing existing official statistics and studying operational problems of public services.

There is also the considerable support to social science research given by the major charitable foundations, but Mr. Freeth thought that this source of support is likely to diminish in future. He believes that much more work in this field is needed in the universities and colleges of technology, and hopes that those interested in strengthening the study of the social sciences there will give evidence to the Robbins Committee.

In welcoming Mr. Freeth's reply, Mr. Peart expressed the opinion that a research council for the social sciences should be created, and hoped that the Government would act after considering the views of the Council for Scientific and Industrial Research and the recommendations of the Robbins Committee. Referring particularly to the need for more educational research to provide the basis of objective advice for decisions, he instanced the value of a proper survey into the way in which comprehensive schools are meeting educational needs and fitting into local environment, and of a proper social survey of university education, embracing the place of students, the nature and length of courses and the wastage of resources. It is as a stimulus to social research in the universities, to undergraduate and postgraduate training in this field and the provision of field observation groups and group behaviour studies in a wide field of human activity in industry as well as detailed socioclinical studies and surveys that Mr. Peart thinks that a social research council would help to supply the detailed information needed as a basis for sound decisions by the Government in matters which are often non-political.

B.B.C. ENGINEERING MONOGRAPHS

IN 1955, the British Broadcasting Corporation began the issue of a series of B.B.C. Engineering Monographs*, describing technical work carried out by the Engineering Division of the Corporation in the development and operation of their sound and television broadcasting services (*Nature*, 177, 519; 1956). Nineteen of these monographs had been issued up to June 1958 (*Nature*, 182, 1490; 1958); and a further eighteen have been published in the following three years, up to June 1961. The authors of these monographs are usually members of the staff of the various departments of the Engineering Division of the Corporation, and collectively they show the progress of the development of the service of sound and television broadcasting through the Research, Designs and Planning and Installation Departments to the practical operation of the service transmitting stations.

The range of subjects dealt with is indeed wide. On the sound-broadcasting side, the problems of programme switching, control and monitoring have been described, together with details of the methods used to check the quality of very high frequency broadcasting. The various methods which can be used for stereophonic broadcasting have been surveyed and discussed with the view of appreciating the possibilities of this technique for domestic reception. In connexion with television, the subjects

* B.B.C. Engineering Monograph No. 34: A Mobile Laboratory for U.H.F. and V.H.F. Television Surveys. E. W. Taylor and L. C. Munn. Pp. 16. No. 36: Some Aspects of Optical Lens Performance. Part 1: Secondary Flare in Lenses. By K. Hacking. Part 2: The Design of Lens Hoods. By W. N. Sproson. Pp. 19. No. 37: An Instrument for Measuring Television Signal-to-Noise Ratio. S. M. Edwardson. Pp. 23. (London: British Broadcasting Corporation, 1961.) 5s. each.

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dealt with in recent monographs, range from the performance of optical lenses in the cameras, the general testing of cameras, and the processing of films, to the equipment of television studies and a new survey of experimental colour transmissions.

Progress common to both sound and vision broadcasting is reported in such subjects as the design of transmitting aerial systems and the general study of radio wave propagation. This work is essential in assessing the coverage likely to be attained from new transmitting stations, and particularly from those which will be required for extended services in the future. Some of the research work is carried out with mobile laboratories, while the operational engineers use mobile studios and control rooms for their outside sound and television services. The latest types of these mobile units are described in separate monographs.

Altogether these publications show that the B.B.C. is well aware of its responsibility to keep the technical side of its services up to date, and to take full advantage of progress and developments, whether these arise from within its own organization or from elsewhere in the fields of radio research and application.

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

THE report of the Governing Body of the Lister Institute of Preventive Medicine for 1961* again contains a detailed record of the numerous investigations carried out in the various departments.

In the Department of Microbiology, for example, the Guinness–Lister Unit continued its exploration of bacterial genetics, mainly of the *Salmonella* bacilli. The problems investigated include conjugation and gene-transfer initiated by the colchicine type of fertility factor and by a mutant of the classical fertility factor, *F* of Hayes; the effect of a colchicine factor on the resistance to ultra-violet irradiation; the genetics of resistance of *E. coli* to colchicines; the genic control of flagellation in a number of *Salmonella* mutants and of the production of different varieties of flagellar protein; and the chemistry of flagellar proteins and of the cell-wall polysaccharides.

The rates of bacterial multiplication in the infected mouse were studied with pathogenic *Salmonella* marked either with a non-replicating prophage or with an abortively transduced gene from another strain of *Salmonella*. The other purely microbiological investigation concerns the antigenic analysis of certain free-living protozoa.

In researches concerned with the immunology and pathology of infective diseases, the Institute's work includes infections by viruses, pleuropneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in The Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group. Laboratory work at the Institute includes growth of trachoma and inclusion conjunctivitis virus in tissue culture, serological classification of the viruses, the vaccination of baboons against inclusion conjunctivitis and the serology of Gambian *Haemophilus* strains. In the smallpox-vaccine department, there is continued progress towards making vaccine from virus grown in tissue culture. The properties of measles virus were also studied.

As an indirect means of determining the significance of antibody to pleuropneumonia-like organisms in human subjects with abacterial urethritis and other kinds of genital infection, the study of the relation of pleuropneumonia-like antibody to the infecting agent and stage of infection in rats and mice was continued.

The immunological study of bacterial infections includes an analysis of the action of two types of protective antibody in mice infected with the whooping-cough bacillus; the biological activity of the *C* polysaccharide of *Streptomyces pyogenes*; and the process of refinement of antitoxin by proteolysis.

Further investigation has been made of the immunology of experimental trypanosomiasis in terms of soluble and bound immunizing antigen as well as of methods of bulk growth of the trypanosomes for antigenic analysis.

The refined serological methods devised to identify the animal source of food for blood-sucking insects provides valuable facts about the feeding-habits of tsetse flies in regions where these insects are vectors or possible vectors of disease.

In the Department of Biochemistry, the researches concerned were again with three kinds of substances, namely, the blood group substances, the cellular phospholipids and starches.

The investigations of the substances of the *ABO* blood-group system include analysis by improved methods of partial hydrolysis, determination of the amino-acid moiety of the substances and its relation to serological specificity, transformation of blood-group specificity by the action of selected enzymes and measurement of the molecular properties of the substances.

Work on the structure of phospholipids, their distribution in the tissues, cells and cellular particles, and their possible function in the cell includes analysis of the phospholipids of *Micrococcus lysodeikticus* of the pig kidney, lung and spleen and of mammalian plasmalogens.

The enzymes of plants, animals and bacteria synthesizing and degrading starch and glycogen were studied with regard to their specific effects on natural carbohydrates and on modified carbohydrates produced by chemical methods, including starches and various glucose phosphates. A disaccharide other than isomaltose was obtained from a dextran, and a new trisaccharide. Carbohydrates were synthesized for use as haptens in the immunochemical analysis of dextrans.

With human and animal plasma proteins, the Institute's work is concerned with the isolation, refinement, characterization, assay and, in some cases, clinical trial of the various biologically active proteins of human plasma.

A reliable assay of human antihæmophilic globulins was devised and improvements made in separation of stable preparations of the globulins from plasma.

Work on the isolation and characterization of biologically active proteins include those of plasmin, plasminogen, albumin, caeruloplasmin and Christmas factor.

* Lister Institute of Preventive Medicine. Report of the Governing Body, 1961. Pp. 29. (London: Lister Institute of Preventive Medicine, 1961.)

ISOELECTRONIC PRINCIPLE, THERMOCHEMISTRY, AND REACTIVITY OF RELATED FREE RADICALS

By Dr. PETER GRAY and J. C. J. THYNNE
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THE isoelectronic principle states that molecules containing identical numbers of valence electrons show many similarities in physical properties, molecular symmetry, structure and chemical reactivity. The principle was originally based on the striking physical resemblance between such isoelectronic molecules as nitrogen and carbon monoxide or nitrous oxide and carbon dioxide. It may also be applied to free radicals. Moreover, its range of usefulness is widened by the possibility of substitution of isoelectronic groups (such as $—CH_2—$, —NH— and —O—) for one another.

Recently, attention has been directed[1] to the usefulness of the isoelectronic principle in correlating the patterns of reactivity of two families of free radicals, namely, the alkyl radicals RCH_2· and the alkoxy radicals RO·. For example, the parallelism between their decomposition reactions $RCH_2CH_2· \rightarrow R· + C_2H_4$ and $RCH_2O· \rightarrow R· + CH_2O$ has been discussed. In general it is to be expected, if two free radicals are isoelectronic, that they will exhibit similar patterns of reactivity. An important qualification should be made, however. It is well known that thermochemistry plays an especially important part in determining the behaviour and reactivity of free radicals. If a certain free radical may react by either of two paths, then their relative thermochemical requirements may be at least as important a consideration as a comparison with the behaviour of an isoelectronic analogue. When the isoelectronic principle and thermochemistry reinforce one another's influences, then prediction of the behaviour of the radical is probably safe. When they oppose one another, which will be dominant is not known.

Accordingly, in this article, we are concerned with the thermochemistry and the reactivity of two isoelectronic pairs of radicals: (i) the acyloxyl ($RCO_2·$) and the azoxyalkyl radicals ($R\dot{N}_2O$), and (ii) the alkoxycarbonyl ($RO\dot{C}O$) and the alkoxyazo radicals ($RONN\dot{}$). These radicals, it will be noticed, also form two isomeric pairs (iii) $RCO_2·$ and $RO\dot{C}O$, and (iv) $R\dot{N}_2O$ and $RONN\dot{}$; so that comparison may be made not only between the behaviour of isoelectronic radicals but also between the behaviour of isomeric radicals.

(1) Acyloxyl and azoxyalkyl radicals, $RCO_2·$ and $R\dot{N}_2O$. Of these families of radicals the acyloxyl radicals are the most well known. They may conveniently be prepared[2] by the thermal decomposition of diacyl peroxides. The acyloxyl radicals readily decompose yielding carbon dioxide together with an alkyl radical (reaction 1), and it is this fact that has resulted in the frequent use[3,4] of diacetyl peroxide as a low-temperature source of methyl radicals:

$$R-C(=O)-O\cdot \longrightarrow R· + CO_2 \qquad (1)$$

Isoelectronic with the acyloxyl radicals are the isomeric pair of azoxyalkyl radicals $R—N=N·$ (O below) and $R—N=N·$ (O below). (These are related to the azoxyalkanes RN_2OR in the same way as the acyloxyl radicals $RCO_2·$ are related to the esters RCO_2R). Although aliphatic azoxy-compounds[5] have been prepared, their possible use as a source of radicals does not appear to have been much investigated, perhaps because of the hazards involved in their use.

Each of these families appears to have only one obvious mode of decomposition: acyloxyl radicals according to reaction 1, and the azoxyalkyl radicals according to reaction 2:

$$R\dot{N}_2O \longrightarrow R· + N_2O \qquad (2)$$

(2) Alkoxycarbonyl and alkoxyazo radicals, $RO\dot{C}O$ and $RONN\dot{}$. The alkoxycarbonyl radicals ($RO\dot{C}O$) have, until recently, been less well studied than the acyloxyl radicals although they are of considerable interest kinetically. They can be generated by the alkyl radical-induced decomposition of alkyl formates[6] (reaction 3), and this reaction has been shown[7] to be a good general source of thermalized alkyl radicals:

$$R' + H\,COO\,R \longrightarrow R'H + \cdot C(=O)—O\,R \qquad (3)$$

Isoelectronic with these radicals are the alkoxyazo radicals $RONN\dot{}$. These may be prepared by the thermal decomposition of hyponitrite esters[8,9] (reaction 4).

$$RON = NOR \longrightarrow RONN\dot{} + RO· \qquad (4)$$

Comparison of the two isoelectronic families $RO\dot{C}O$ and $RONN\dot{}$ is more subtle than that of the radicals $RCO_2·$ and $R\dot{N}_2O$, because, in principle, each may undergo either of two decompositions:

$$RO\dot{C}O \rightarrow RO· + CO \qquad (5a)$$
$$\rightarrow R· + CO_2 \qquad (5b)$$
$$RONN\dot{} \rightarrow RO· + N_2 \qquad (6a)$$
$$\rightarrow R· + N_2O \qquad (6b)$$

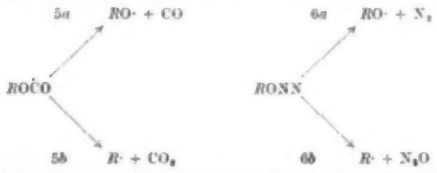
In fact, the observed decomposition behaviour is clear-cut and different in the two families. Alkoxycarbonyl radicals yield only alkyl radicals and carbon dioxide, for in none of the experiments[6,7,15] on the radicals $RO\dot{C}O$ ($R = CH_3$ to $R = C_4H_9$) is any product characteristic of alkoxy radicals found. Alkoxyazo radicals produce only alkoxy radicals and nitrogen. Neither in the decomposition of the lower alkyl hyponitrites[8] in the gas phase nor of benzyl hyponitrite[9] in solution is nitrous oxide or any product compatible with the formation of alkyl radicals found.

This striking difference is a departure from the behaviour expected on the basis of the isoelectronic

Table 1. Energy Requirements of Alternative Decomposition Paths for Related Radicals $RO\dot{C}O$ and $RO\dot{N}_2$

R	$\Delta H_{5b} - \Delta H_{5a}$ (kcal. mole^{-1})	$\Delta H_{6a} - \Delta H_{6b}$ (kcal. mole^{-1})
CH_3	35·7	51·5
C_2H_5	34·7	52·5
$n.C_3H_7$	32·7	54·5
$i.C_3H_7$	35·7	51·5
$n.C_4H_9$	31·6	56·5

$\Delta H_{5b} - \Delta H_{5a}$, enthalpy difference between reaction paths 5*b* and 5*a*; $\Delta H_{6a} - \Delta H_{6b}$, enthalpy difference between reaction paths 6*a* and 6*b*.



principle. Since it is essentially a kinetic difference it is helpful to consider the contribution of the Arrhenius factor A and the activation energy E to the velocity constant of decomposition. It is difficult to see any real basis for predicting different A factors for unimolecular decomposition of the radicals $RO\dot{C}O$ and $RONN\dot{}$, and it may be considered that the difference is attributable to difference in activation energies. In turn, the origin of the differences in activation energy may be sought in the thermochemistry of the radicals. Table 1 summarizes the differences in the energy requirements between the two decomposition paths for some members of each family of radicals. Thus in each family the energetically favoured path is followed. While carbon dioxide formation from the alkoxycarbonyl radicals needs some 35 kcal. less than carbon monoxide formation, nitrous oxide formation from the alkoxyazo radicals requires some 50 kcal. more for the production of nitrogen.

It is worthy of comment that the differences in energy requirements of the individual reaction paths of each radical can be evaluated without knowledge of the heats of formation of the individual radicals; thermochemical data for the reaction products are sufficient.

(3) Thermochemistry and reactivity of the acyloxyl and alkoxycarbonyl radicals. Although, for the purpose of comparison above, values of the heats of formation of the radicals $RO\dot{C}O$ are not needed, they are needed to make a comparison of the isomers $RO\dot{C}O$ and $R\dot{C}O_2$. Enthalpies of formation for some of the lower acyloxyl radicals are known[10] and these are tabulated (col. 2, Table 2). Values of enthalpies of formation may also be obtained for free radicals by group-energy terms[11], using a value of -39 kcal. for $H(\dot{C}O_2)$. These values also appear in col. 2. They have been used to calculate the enthalpy change of the decarboxylation of acyloxyl radicals, and these results appear in col. 3, Table 2.

No experimentally obtained thermochemical data exist for the alkoxycarbonyl radicals. Values for $\Delta H_f(RO\dot{C}O)$ which are unlikely to be seriously in error can be inferred from kinetic work on hydrogen abstraction by alkyl radicals for a series of aldehydes[12] (RCHO) on one hand, and from various formate esters[14] (RO.CHO) on the other. In general, the values of the activation energies of hydrogen abstraction for both aldehydes and formates differ by little more than 1–2 kcal. mole^{-1} for members of both series, suggesting that the groups R and RO (which are not immediately adjacent to the point of radical attack) have little effect on the strength of the C—H bond being broken. If these bonds have similar strengths in the two families, then:

$$D(H - \overset{\overset{O}{\|}}{C}.CH_3) \simeq D(H) - \overset{\overset{O}{\|}}{C}OCH_3) \simeq 82 \text{ kcal.}$$

Since:

$$D(H - \overset{\overset{O}{\|}}{C}OCH_3) = \Delta H_f(H) + \Delta H_f(COOCH_3) - \Delta H_f(HCOOCH_3)$$

then a value of $-53{\cdot}6$ kcal. mole^{-1} may be calculated for the heat of formation of the methoxycarbonyl radical. Thermochemical data exist for few formates; however, it is still possible to obtain values for the heats for formation of the radicals from thermochemical data[15] for the acetate esters. In this case a similar assumption of equivalence of bond dissociation energies is made, this time comparing the appropriate bond-strengths of acetone and the acetates, using a 'medium' value for $CH_3 - COCH_3$, that is:

$$D(CH_3) - \overset{\overset{O}{\|}}{C}.CH_3) \simeq D(CH_3 - \overset{\overset{O}{\|}}{C}.OCH_3) \simeq 79 \text{ kcal.}$$

Using this bond energy, a value of $-51{\cdot}7$ kcal. mole^{-1} is obtained for $\Delta H_f(CH_3O\dot{C}O)$ which is in reasonable agreement with the value of $-53{\cdot}6$ kcal. obtained using methyl formate as the radical source. Values of ΔH_f for other alkoxycarbonyl radicals obtained in this manner appear in col. 4, Table 2, and using these values we may now make a direct comparison of the acyloxyl and alkoxycarbonyl radicals.

Assigning a value of $-18{\cdot}5$ kcal. to $H(\dot{C}O)$, then by using the usual group-energy terms, values of $\Delta H_f(RO\dot{C}O)$ may be calculated. These values are tabulated in col. 5, Table 2, and are in quite good agreement with the values obtained using the heats of formation of the acetate esters (col. 4).

Table 2. Enthalpies of Formation and Bond Dissociation Energies of Acyloxyl and Alkoxycarbonyl Radicals
All values in kcal. mole^{-1}

R	$\Delta Hf\ R\dot{C}O_2$	$D(R—CO_2)$	$\Delta Hf\ RO\dot{C}O$	$\Delta Hf\ RO\dot{C}O$	$D(RO—CO)$	$D(R—OCO)$
CH_3	-45^a	$-17{\cdot}5$	$-51{\cdot}7$ ($-53{\cdot}6$ from $HCOOCH_3$)	$-55{\cdot}8$	24·8	$-10{\cdot}9$
C_2H_5	-54^a	$-15{\cdot}5$	$-59{\cdot}6$	$-60{\cdot}8$	24·7	$-10{\cdot}0$
nC_3H_7	-60^a	-13	$-65{\cdot}8$	$-65{\cdot}8$	26·4	$-6{\cdot}3$
iC_3H_7	-60^b	$-16{\cdot}7$	$-68{\cdot}9$	$-67{\cdot}0$	27·5	$-8{\cdot}2$
nC_4H_9	-64^b	$-12{\cdot}2$	$-71{\cdot}9$	$-70{\cdot}8$	28·5	$-3{\cdot}2$
			^{e}	^{d}	^{e}	^{e}

a, Values reported by Jaffé, Prosen and Szware (ref. 10); *b*, calculated from group energy terms ($H(CO_2\cdot) = -39$ kcal.; *c*, obtained using $D[R—COOR] = 79$ kcal., and using heats of formation of acetates (ref. 15); *d*, calculated from group energy terms $H(CO) = -18{\cdot}5$ kcal.; *e*, values of $\Delta Hf\ (RO\dot{C}O)$ taken from col. 4.

(4) Energetics of decarboxylation of $R\dot{O}CO$ and $R\dot{C}O_2$. Since:

$$D(CH_3O - CO) = \Delta H_f(CH_3O) + \Delta H_f(CO) - \Delta H_f(CH_3O\dot{C}O)$$

and

$$D(CH_3 - OCO) = \Delta H_f(CH_3) + \Delta H_f(CO_2) - \Delta H_f(CH_3O\dot{C}O)$$

then, using a value of $-$ 51·7 kcal. for $\Delta H_f(CH_3O\dot{C}O)$, we obtain:

$$D(CH_3 - OCO) = - 10{\cdot}9 \text{ kcal. mole}^{-1}$$

and

$$D(CH_3O - CO) = 24{\cdot}8 \text{ kcal. mole}^{-1}.$$

Similar values for other alkoxycarbonyl radicals appear in columns 6 and 7 of Table 2. Decarboxylation of the $R\dot{O}CO$ radicals, although involving bond fission, is always exothermic (ΔH is negative): thus the alkoxycarbonyl radicals are thermodynamically unstable with respect to decarboxylation. This is the same rare behaviour that is observed in the acyloxyl radical where, for instance, $D(CH_3 - CO_2) = - 17{\cdot}5$ kcal. Throughout the two series, the alkoxycarbonyl radicals are thermodynamically less unstable than their isomers.

(5) Stability and reactivity of the $R\dot{O}CO$ and $R\dot{C}O_2$ radicals. There has been some controversy[16,17] about the kinetic stability of the acetyloxyl radical. The discussion has centred on whether the acetyloxyl radical survives long enough to react with a substrate and simultaneously decarboxylates, or whether the radicals are decarboxylated so that the substrate is attacked only by the methyl radicals produced. Recently, Herk and Szwarc[17] have concluded that, even in the liquid state, the reactions observed with the acetyl peroxide system are due to methyl radicals not to acetate radicals. No methyl acetate has been found in the gas-phase thermal decomposition of the peroxide, suggesting that there the acetyloxyl radical is not stable enough to associate with methyl radicals by:

$$CH_3CO_2\cdot + CH_3 \longrightarrow CH_3\,COO\,CH_3$$

Szwarc and Herk[12] consider that the decarboxylation of the acetyloxyl radical requires some small activation energy, about 1–2 kcal. mole^{-1}.

Less is known about the stability and reactivity of the alkoxycarbonyl radical. It appears from a consideration of the energetics of decarboxylation and from experimental observations[6,14,18] that the radical is unstable. Kebarle and Lossing[19], however, found traces of methyl acetate in a mass-spectrometric study of the mercury-photosensitized decomposition of methyl formate, presumably produced by the association of methyl with methoxycarbonyl radicals, that is:

$$CH_3 + CH_3OCO \longrightarrow CH_3\,COO\,CH_3$$

This apparent stability would suggest that the activation energy for decomposition of the methoxycarbonyl radical is perhaps somewhat greater than the value of 1–2 kcal. suggested for the acetyloxyl radical, a conclusion which would also concur with the relative bond strengths we have calculated for CH_3CO_2· and $CH_3O\dot{C}O$ ($-$ 17·5 kcal. and $-$ 10·9 kcal. respectively).

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PATHS OF INTER-MOLECULAR ENERGY TRANSFER IN PHOTO-SENSITIZED OXIDATION-REDUCTION

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IT has been recognized that there are two types of energy transfer of the electronic excitation from sensitizer to energy acceptor; one corresponds to the case of weak resonance coupling owing to the transitional dipole–dipole interaction between photo-excited sensitizer and unexcited acceptor, the other is the case of strong resonance coupling for which the effect of the first order of the same interaction as above is responsible.

The problem in this article is confined to the solution system of organic dyes. When S and A refer to the sensitizer and the energy acceptor, respectively, the types of energy transfer mentioned here are written in the manner of a chemical reaction as follows:

$$h\nu_s + S + A \rightarrow S^* + A \rightarrow S + A^* \rightarrow A'$$
$$\uparrow \qquad\qquad \downarrow$$
$$\longrightarrow \text{redox process} \quad (a)$$
(+ reductant (or oxidant))

$$h\nu_s + S + A \rightarrow S^* + A \rightarrow [S \ldots A]^* \rightarrow S + A'$$
$$\uparrow \qquad\qquad \downarrow$$
$$\longrightarrow \text{redox process} \quad (b)$$
(+ reductant (or oxidant))

where $h\nu_s$ denotes the energy of a photon absorbed by S, the molecular species accompanied with asterisk mean the photo-excited molecules in the singlet state, and A' stands for the metastable A in the triplet state. Here we have written the resonance state as $[S \ldots A]^*$. In other words, this state can be described as the resonance between the states (S^*, A) and (S, A^*). The case of (a) is called the weak resonance coupling, and the case of (b) is called the strong resonance coupling.

The following criteria, which were appointed by Simpson and Peterson[1] in the problem of the electronic spectra of molecular crystals, serve as an answer for

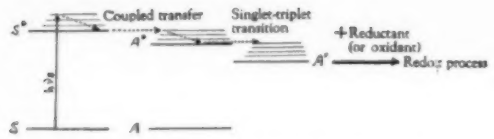


Fig. 1. Simplified diagram of sensitization in the case of weak resonance coupling

why either type (*a*) or (*b*) can be found in the mechanism of sensitization:

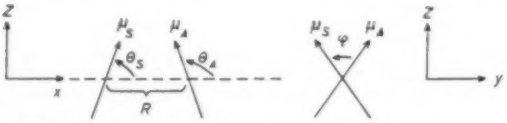
$$2V/\Delta \ll 1 \text{ for the case of weak coupling} \qquad (a')$$
$$2V/\Delta \gg 1 \text{ for the case of strong coupling} \qquad (b')$$

where V represents the so-called resonance coupling parameter, which is defined by the matrix element of the electronic dipole–dipole interaction, and Δ is the width of the band related to the electronic transition under consideration.

In the former case, it can be said that the energy transfer is achieved with the transition from the initial state $\psi(S^*)\psi(A)$ to the final state $\psi(S)\psi(A^*)$ owing to the dipole–dipole interaction, and the transfer probability has the dependence R^{-6} on the intermolecular distance R, where $\psi(S)$,$\psi(S^*)$, and $\psi(A)$, $\psi(A^*)$ are the electronic wave functions of the unexcited and excited S and A, respectively. In the present problem, the resonance coupling parameter becomes:

$$V = -\frac{1}{\varkappa R^3}\int\psi(S^*)\psi(A)\mu_S\mu_A\psi(S)\psi(A^*)\mathrm{d}\tau_S\mathrm{d}\tau_A \times (2\cos\theta_S\cos\theta_A - \sin\theta_S\sin\theta_A\cos\varphi) \qquad (c)$$

where $\varkappa$ is the dielectric constant of the medium, μ_S and μ_A are the dipole moments referring to S and A, and the angles of relative orientation of the dipoles are defined as:



If S and A are parallel in the resonance state, $\theta_S = \theta_A = \frac{\pi}{2}$ and $\varphi = 0$. Hence, V can be written as:

$$V = \mu_S'\mu_A'/\varkappa R^3 \qquad (d)$$

where μ_S' and μ_A' are the transitional dipole moments given by $\mu_S' = \int\psi(S^*)\mu_S\psi(S)\mathrm{d}\tau_S$ and $\mu_A' = \int\psi(A)\mu_A\psi(A^*)\mathrm{d}\tau_A$, respectively. It goes without saying that the transition probability of the energy transfer in weak resonance coupling is proportional directly to the square of equation (*c*) (ref. 2). The energy transfer from S to A is succeeded by the non-radiative transition from the excited singlet state to the triplet state due to the spin-orbit coupling in the acceptor molecule. The metastable molecular species of the produced triplet state take part in redox processes with a reductant or oxidant. This is the mechanism of photo-sensitized redox reaction in the case of sensitization due to the weak coupling. A simplified energy diagram of this mechanism is shown in Fig. 1.

The example can be found in the following system: in the ethanol solution of S = uranin, A = methylene blue and reductant = ascorbic acid or phenylhydrazine, the bleaching reaction of A photo-sensitized by S occurs with photo-excitation of S (ref. 3).

A characteristic property of the sensitization in the case of weak resonance coupling can be seen in the fact that the vibrational motion in the excited electronic state continues during an interval of time of $10^{-9} \sim 10^{-8}$ sec. (that is the life-time of fluorescence) according to the inequality (*a'*).

On the other hand, in the case of strong resonance coupling the resonance effect of the first order of interaction must be taken into account by taking the following linear combinations of both states $\psi(S^*)\psi(A)$ and $\psi(S)\psi(A^*)$ at a fixed molecular configuration:

$$\psi_+ = 2^{-1/2}\{\psi(S^*)\psi(A) + \psi(S)\psi(A^*)\} \qquad (e, \text{i})$$
$$\psi_- = 2^{-1/2}\{\psi(S^*)\psi(A) - \psi(S)\psi(A^*)\} \qquad (e, \text{ii})$$

because the electronic coupling occurs before the vibrational motion in the degenerate excited state at a reasonance configuration; this is the meaning of the inequality (*b'*). The resonance split of energy is given by the difference between the energy-levels of ψ_+ and ψ_-, or by $2|\mu_s'||\mu_A'|/\varkappa R^3$. Provided that $\vec{\mu_s'} = -\vec{\mu_A'}$, the lower energy state is ψ_+ and the higher one is ψ_-. Moreover, it is important that the dipole transition from ψ_+ to $\psi(S)\psi(A)$, in which $\psi(S)\psi(A)$ is the ground state free from interaction, is forbidden owing to vanishing the total transitional dipole moment.

Because the energy-level of the state ψ_+ is lower by $|\mu_s'|^2/\varkappa R^3$ than the level of the excited state of S without A, we can interpret the state ψ_+ as the excited state stabilized by the transitional dipole–dipole coupling with A. It can be expected that the transition probability from the stabilized singlet state ψ_+ to the lowest triplet state of A in the resonance complex is much greater than the transition probability from the excited singlet state of A only to the same triplet state, because the stabilizing energy, or $|\mu_s'|^2/\varkappa R^3$, is comparable with the energy of separation between the first excited singlet state and the lowest triplet state of A itself; in many cases of organic dyes the energy is a few thousand cm.$^{-1}$ (refs. 4 and 5). After the transition of A to the triplet state, the resonance complex $[S \ldots A]^*$ dissociates repulsively, owing to the vanishing of the transitional dipole moment. The metastable molecule A' in the triplet state proceeds to redox process with a reductant or oxidant. These processes are shown diagrammatically as Fig. 2.

Although it is not the example of sensitization in a narrow sense, an interesting example of the latter case can be found in the photo-reduction of some organic dyes in an aqueous solution of high polymer; in this reaction it is important to produce the metastable species of the dye due to the interaction between the photo-excited and unexcited dyes in the bound state with high polymer[6]. Another example is the reduction of crystal violet photo-sensitized by trypaflavine in the aqueous solution. As is well known, the triphenylmethane dyes cannot be photo-reduced by ascorbic acid or some other reductant in

→, radiative transition; - - →, radiationless transition

Fig. 2. Simplified diagram of sensitization in the case of strong resonance coupling

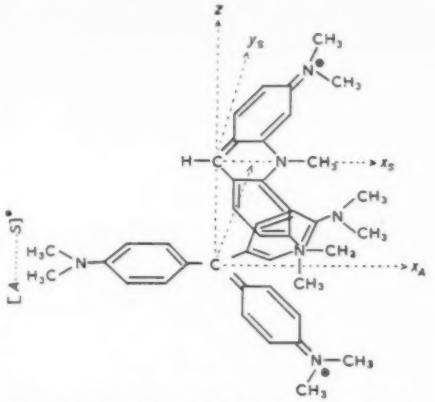


Fig. 3. A possible configuration in the resonance state of trypaflavine (*S*) and crystal violet (*A*)

free solution except in the presence of high polymer which contains the carboxyl groups. However, the photo-reduction of the dye with ascorbic acid occurs when trypaflavine is used as sensitizer[7]. The mechanism can be explained by sensitization of the latter case. In the resonance state in which the excited trypaflavine interacts with the unexcited crystal violet, the molecular plane surface of crystal violet will be conserved as a result of resonance stabilization. A possible molecular configuration in the resonance state is shown in Fig. 3. The excitation energy absorbed by crystal violet in the free system from trypaflavine, or even in the presence of trypaflavine, does not activate the redox reactivity, because the dissipative processes predominate over the intramolecular transition to the triplet state due to the deficiency of the stability of the excited singlet state of crystal violet.

The experimental facts show that the metastable crystal violet produced after the resonance stabilization in the sensitization takes part in the redox process with ascorbic acid, and the stabilization of the excited crystal violet results also from the resonance interaction with the unexcited crystal violet in the presence of high polymer.

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ELECTRON MICROSCOPIC OBSERVATIONS OF STRUCTURE IN ULTRA-THIN SECTIONS OF VITRINITE IN COAL

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MOST electron microscopic examinations of coal have been made on replicas of polished, etched, or natural fracture surfaces[1–4]. Faithful reproduction of structures in such replicas may be questioned, and resolution of detail is inferior to that which may be expected in direct observation of coal specimens. Consequently, the preparation of coal sections thin enough to transmit electrons has been an important objective. Recently, workers at the Centre d'Etudes et Recherches des Charbonnages de France[5] have succeeded in cutting ultra-thin sections of soft coals, using a microtome and a diamond knife. We have adopted their procedure and succeeded in cutting sections, 500–2000 Å. thick, of coals of various ranks and hardness. Success in preparing such sections has opened up a very promising field of study involving ultra-fine structures in coals such as preserved plant cell components, pores, and possibly structural units or crystallites.

Ultra-thin sections of coal are cut with a diamond knife mounted on a microtome designed for preparing sections for electron microscopy. The sections are cut from an 8–14 mesh particle embedded in a polymerized mixture of equal volumes of methyl and butyl methacrylates. They have an area of approximately 1 mm.^{2}. When cut the sections float out on a liquid surface from which they are picked up on specimen screens. A detailed description of the technique of preparing these sections will be presented elsewhere[6].

The limited number of electron microscopic observations of ultra-thin sections of vitrinites made thus far have revealed interesting structural details. Interpretation of these details has been approached

Fig. 1. Electron micrograph of structure in an ultra-thin section of a semi-anthracite (×*c.* 7,500)

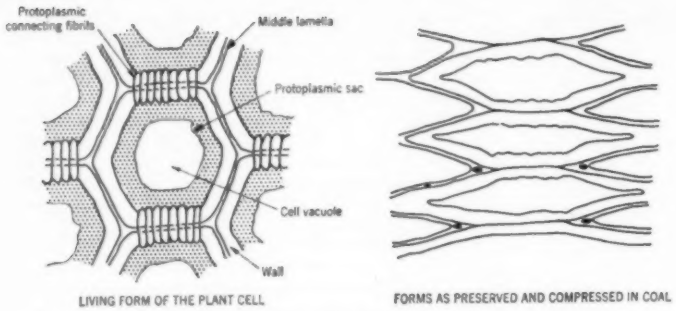

Fig. 2. Cell structure in the living plant cell and typical form of preservation in coal

cautiously because of possibilities of artefacts introduced in section preparation. One type of structure is shown in Fig. 1. This is an electron micrograph of a relatively thick section (≈2000 Å.) of a semi-anthracite. It shows outlines of areas resembling cross-sections of cells, with little internal detail visible. These areas have dimensions of about $3 \times 10\mu$. The manner in which such a cell structure might be preserved in coal is illustrated in Fig. 2[7]. Compression of the original plant cell form can result in the type of structure shown, to which the areas in Fig. 1 bear a striking resemblance. Such structures have been seen in electron micrographs of replicas of etched coal surfaces[1], but the dimensions were about $5 \times 50\mu$. Turner[8] has shown cells in anthracite averaging about $16 \times 33\mu$, and Raistrick and Marshall[9] have shown relatively uncompressed cells, in coals of unspecified rank, ranging upwards from 30μ in diameter. Thus, the areas of Fig. 1 seem to be considerably smaller than the normal cell sizes seen in coal. However, these differences in size are possible and the pattern is so well-defined that it probably represents an authentic structure. Structures of this kind appear to become prominent in the thicker ranges of these ultra-thin sections. This may be due to distortion of the structure because of compression in the thinner sections or to greater contrast in the thicker ones.

Another kind of structure similar to that of Fig. 1 is shown in Fig. 3. This was seen in a section of high-volatile *A* bituminous coal from the Pittsburgh bed. Here, too, there are some of the characteristics of a cell structure. These units average about $\frac{1}{2} \times 2\mu$, about a fifth as large as those in Fig. 1. Variations of this pattern also appear in several other coals. The appearance of this structure may have been determined largely by stresses operating during the cutting process. However, even if this were the case, the strains contributing to this pattern must have been conditioned by some repetitive inhomogeneity in the coal substance, for this pattern is not always obtained, it does not always cover the whole section, and it is not restricted to the middle or to the edges of the sections. It may be premature to accept the observed pattern as a definite structure; nevertheless, it could be regarded as an indication of some basic periodicity in the coal make-up.

Another type of structure seen in several vitrinites of different rank is shown in Fig. 4. This appears to be a very fine fibrillar structure with recurring units less than $0{\cdot}1\mu$ in width. A fibrillar structure similar to, but coarser than, this has been seen in an ultra-violet light photomicrograph of a delignified cell wall of western hemlock[10]. It has been suggested that the cell walls of wood consist of interpenetrating systems of cellulose and lignin. Although coal is believed to contain little cellulose, it is possible that the fibrillar structure of wood is preserved in coal. However, this apparent structure may be an artefact introduced in the section during the cutting process. Its prevalence throughout the sections of this coal and several other coals favours this suspicion. An authentic interpretation must await further examination.

Fig. 3. Electron micrograph of structure in an ultra-thin section of a high-volatile *A* bituminous coal (×*e*. 7,500)

Fig. 4. Electron micrograph of structure in an ultra-thin section of a high-volatile *C* bituminous coal (×*e*. 22,500)

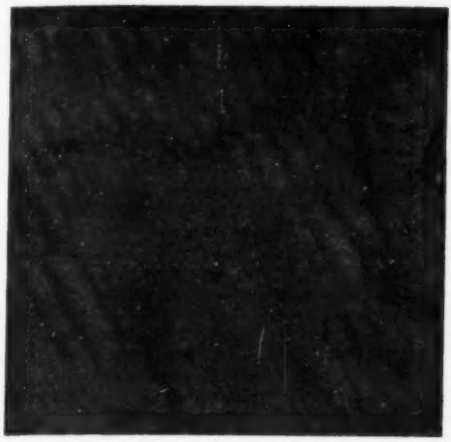

Fig. 5. Electron micrograph of structure in an ultra-thin section of an anthracite (×*c.* 7,500)

Fig. 5 shows an ultra-thin section of an anthracite. Three features are evident in this electron micrograph: an irregular pattern of thin areas surrounding an oval portion with a central thin area, and an overall background of striæ. The significance of the first two features with regard to coal structure is not readily evident, but it seems highly unlikely that the oval area and others similar to it could be preparation artefacts. They may be remnants of resin globules. The striæ, which persist throughout this section, are no doubt similar to those observed in Fig. 4 and, as discussed there, may or may not have structural significance.

It may be readily inferred from the foregoing discussions that interpretations of electron micrographs of these ultra-thin sections are far from conclusive. However, these preliminary results and comments are presented here as a stimulus to further studies of the physical fine structure of coals.

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POISONING AND SIGN REVERSAL OF PERMSELECTIVE MEMBRANES

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WHENEVER the counterions of a permselective membrane are held firmly to the fixed ions of the membrane, its conductivity decreases[1], and as a part of the fixed charge is neutralized the permselectivity of the membrane decreases also. The higher the electric charge of the counterions, the more firmly are they attached to the fixed sites. George, Rosenberg and Potter[2] even found that the transference number of Th^{+4} ions sank below 0·5—that is, the sign of permselectivity was reversed—when a cation-selective membrane was placed between two Th^{+4} solutions. In this experiment the Th^{+4} functioned both as 'poison' and as an indicator of 'poisoning'; it was the reduced mobility of these ions in the membrane that gave rise to low cationic transference.

I wanted to see whether the membrane could be poisoned to an extent that it became reversed even against ions which in themselves were not poisonous to it. Membranes were immersed between two solutions of potassium chloride of different concentration; here the mobility of K^+ and Cl^- was certainly the same. I then added equal amounts of 'poisoning' ions to both sides, so that there was no concentration gradient of these ions between the two sides. They served only to poison the membrane, while the potassium chloride indicated the degree of poisoning. As soon as the total charge of firmly bound counterions equals the sum total of the charge of fixed membrane-ions, the permselectivity of the membrane becomes zero, and, as the charge of bound counterions increases further, the sign of charge of the membrane changes and its permselectivity is reversed.

I worked with membranes made of parchment impregnated and cured with a phenolsulphonic acid-formaldehyde or with *N*-acetic acid-pyridinium-phenolformaldehyde resin. Permselectivity was generally determined by measuring the e.m.f. across the membrane between two potassium chloride solutions of different concentration which contained the same concentration of poison on both sides. In one experiment where permselectivity had been reversed by Th^{+4} ions I confirmed the sign reversal in a Hittorf transference-number experiment in *N*/10 potassium chloride (containing 0·008 *N* thorium chloride) and found a Cl^- transference number of 0·6 instead of nearly zero in pure potassium chloride.

Up to now I have found three ions which induce sign-reversal in these systems: Th^{+4}, $Fe(CN)_6^{-3}$ and $Fe(CN)_6^{-4}$. Equilibration of the systems was slow, ranging from about ½ hr. with $Fe(CN)_6^{-3}$ to about ½ day with Th^{+4}, being slower at low poison concentrations. It was reversible with the complex cyanides but not entirely reversible with Th^{+4}: though the sign of permselectivity changed back to normal in pure potassium chloride, the original e.m.f. value never returned.

Fig. 1 shows the poisoning curves of the *N*/1 : *N*/2, *N*/10 : *N*/20 and *N*/100 : *N*/200 potassium chloride systems with increasing amounts of $K_3[Fe(CN)_6]$. The points where the e.m.f. disappears are at $1·5 \times 10^{-3}$, $1·5 \times 10^{-2}$ and $1·5 \times 10^{-1}$ poison normality respectively. This is exactly proportional to the three potassium chloride concentrations. The close agreement may be partly fortuitous, but it demonstrates that there is a competition between Cl^- and $Fe(CN)_6^{-3}$ for the active sites of the membrane according to the law of mass action. The membrane capacity was 0·55 m.equiv./gm. At the three respective concentrations

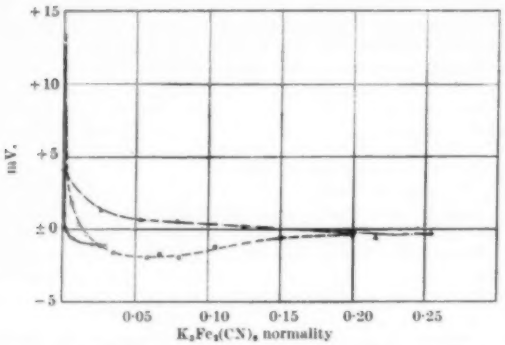

Fig. 1. Changes in e.m.f. due to adjusting the $K_4Fe(CN)_6$ normality on both sides of an anion-selective membrane to the value given by the abscissa

Membranes between 0·010 *N* and 0·005 *N* (○), 0·10 *N* and 0·05 *N* (□) and 1·0 *N* and 0·5 *N* potassium chloride (△)

where the e.m.f. was zero, the membrane was blotted between filter paper and was found to contain 0·63, 0·57 and 0·47 m.equiv./gm. $Fe(CN)_6^{-3}$ respectively. It was not possible to distinguish between 'site-bound' and 'immigrant' counterions, but their concentration within the membrane was high and nearly the same whenever sign reversal occurred.

Permselectivity at high potassium chloride concentration is smaller than at low because of the Donnan equilibrium. The fact that the *N*/10 : *N*/20 curve shows a minimum of −2mV. at 5×10^{-2} poison normality may be due to the fact that progressive addition of the same amount of $K_4Fe(CN)_6$ to both sides decreases the ratio of ionic concentrations between the two sides and it is this ratio which determines the e.m.f. according to the Nernst equation. At high poison concentration the e.m.f. is swamped.

Increasing the potassium chloride concentration on both sides works competitively against poisoning according to Fig. 1. But a reversed e.m.f. may either grow or decrease if the potassium chloride concentration on the concentrated side is increased. It grows in the reversed direction if the poisoning ion is attached so firmly to the resin that only slight competition of potassium chloride occurs. Then the poisoning remains but the ratio of potassium chloride across the membrane increases, and hence the reversed e.m.f. increases also. Two such curves with Th^{+4} as poisoning ion are shown in Fig. 2. On the steeper curve, where the poison concentration and the potassium chloride ratio were both high, the considerable reversed e.m.f. of −19 mV. was attained.

The opposite effect is observed if the competitive influence of potassium chloride overrides its effect on the concentration ratio, as often with the complex cyanides. In such cases, the poison concentration in the membrane evidently decreases on the side towards the more concentrated potassium chloride as more potassium chloride is added, in spite of the fact that the concentration of the poison ion is the same on both sides. The resulting e.m.f. may hence decrease if it was reversed and even change back and increase in the normal direction.

It could be postulated that sign reversal is due to difference in the activity of poisoning ions on the two sides: though their concentration is the same, their activity is less in the more concentrated potassium chloride solution. This difference in activity could override the potassium chloride activities and be the cause of sign reversal. Calculations using the Debye-Hückel formula showed, however, that this hypothesis can be practically excluded for Th^{+4} poisoning and is very improbable with the complex cyanides. Difference in Th^{+4} activity would, for example, account only for a reversed e.m.f. of 0·4 mV. at a point of Fig. 2 where actually 15 mV. was observed. It is certain that the sign of the charge bound to the membrane has been reversed.

Al^{+3}, Fe^{+3}, Cu^{+2}, Ca^{+2}, Mg^{+2}, hexamethonium^{+2}, *N*,*N*-tetramethyl-pyrazolidinium^{+2}, 1,2,3-*tris*-triethylammoniumethoxybenzene^{+3} ('Flaxedil'), HPO_4^{-2} and $O_3P.CH_2.PO_3^{-4}$ ions have been examined also on the same membranes in the same set-up. None of them was sufficiently active as poison to reverse the e.m.f. between *N*/10 : *N*/20 potassium chloride, though Al^{+3} seemed to reach this point within the limits of error (0·2 mV.). Fe^{+3} lowered the permselectivity without reaching zero e.m.f. The other three metal ions and the organic cations were scarcely active at all except that they swamped the e.m.f. by decreasing the ionic ratio. In the experiments of George *et al.*[2], however (that is, without potassium chloride), when only the active ions were present, a sign reversal was observed between *N*/1 : *N*/2 calcium chloride and *N*/1 : *N*/2 magnesium chloride solutions.

It is interesting to note that the three isolated single charges of 'Flaxedil' and the two double charges on the methanediphosphonic ion were not effective, though they gave the total charge of 3 and 4 respectively to their ions. This is probably due to the fact that the charge is spread out in space and not concentrated enough to be active in Coulomb attraction.

The physiological implications of this poisoning and the sign reversal effect are obvious. The small amount of 'poison' necessary to change the potential of, say, a

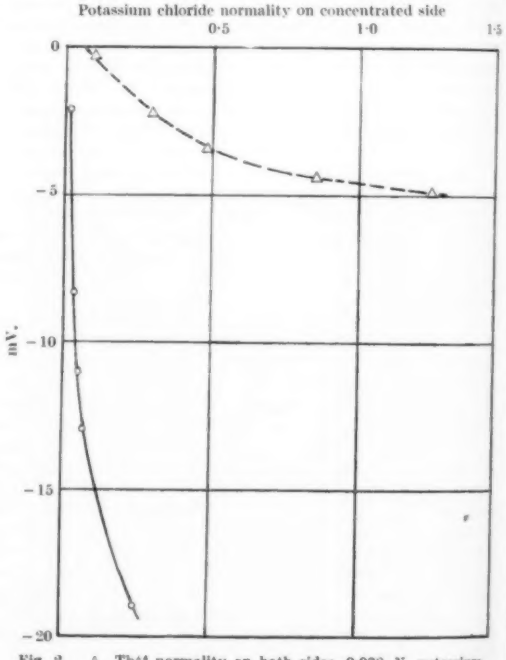

Fig. 2. △, Th^{+4} normality on both sides, 0·038 *N*, potassium chloride normality on dilute side, 0·06 *N*; ○, Th^{+4} normality on both sides, 0·079 *N*, potassium chloride normality on dilute side, 0·006 *N*

nerve ending might be generated by some physiological process and the short circuit could then well travel along the whole fibre. The active volume of such a physiological membrane is very small and the amount of poison necessary very small also, especially bearing in mind that specificity may increase its sensitivity compared with our crude artificial membranes.

I am indebted to Prof. F. Bergmann and to T.E.V.A., Ltd., Jerusalem, for the gift of hexamethonium salts, and to SPECIA S.A., Paris, for the gift of 'Flaxedil'.

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CONTROL OF SPECIFICITY IN HÆMOGLOBIN SYNTHESIS

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In recent years much attention has been focused on the control of specificity in protein synthesis. In general terms it is clear that the ultimate control of specificity resides in the genetic material of the cell. The mechanism whereby the information of the genetic material is translated into the unique amino-acid sequence of the finished protein molecule is generally believed to involve ribonucleic acid. The present article presents results which demonstrate that a particular cell structure, the ribosome, directly controls the amino-acid sequence of the protein which it synthesizes.

Recent work in this laboratory[1–3] has shown with some clarity that in the reticulocyte the site of synthesis of the hæmoglobin molecule is the ribosome. This, together with the large size of the ribosome[4], might suggest that the amino-acid sequence of the hæmoglobin molecule is determined by the structure of the ribosome. The possibility remains, however, that the amino-acid sequence is not determined by the ribosome alone, but by the ribosome acting in conjunction with other cytoplasmic factors. In this respect it is important to note that soluble cytoplasmic factors are known to be implicated in protein synthesis. A specific activating enzyme[5] and a specific soluble ribonucleic acid[5] are thought to be required for the incorporation into protein of each individual amino-acid. Furthermore, it has been suggested[5] that it is a correspondence between the structure of the soluble ribonucleic acid molecule to which the amino-acid is bound, and a specific structural site on the ribosome, which ensures that the amino-acid is incorporated into protein in the correct position. The question to be raised in the present article is whether or not these soluble factors, and in particular soluble ribonucleic acid, play a more direct part in the determination of the amino-acid sequence of hæmoglobin. This work was made possible by the demonstration[1,2,6] of the synthesis of soluble hæmoglobin in a reticulocyte cell-free system.

A first approach to the question was made using ribosomes and soluble fractions from reticulocytes of two species, rabbits and mice. The *C57BL/6J* inbred mouse strain shows a single hæmoglobin fraction by chromatography on 'Amberlite *CG*-50' type II resin with the sodium phosphate buffer number 2 of Allen *et al.*[7]. Under the same conditions rabbit hæmoglobin is strongly bound to the resin, but can be eluted with a two-fold concentration of the same buffer. Thus almost quantitative separation of these two hæmoglobins is possible.

Reticulocytosis was induced in rabbits as described elsewhere[1,3]. Mice were made reticulocytic by the subcutaneous injection of 0·1 ml. of 0·8 per cent phenylhydrazine daily for six days. Twice-washed ribosomes and supernatants were prepared from reticulocyte lysates as described elsewhere[1,3]. Rabbit ribosomes (40 mgm. dry weight) were incubated with 0·6 μmole of uniformly labelled ^{14}C-L-leucine (7 mc./mmole) in a total volume of 8·4 ml. under conditions described[1,3] except that isolated soluble ribonucleic acid and enzyme were replaced by a mixture of 30 mgm. each of the soluble supernatants of both species. After 40 min. of incubation the ribosomes were removed by centrifugation and a hæmoglobin fraction isolated[2] from the supernatant. On chromatography on *CG*-50, 61 per cent of the radioactivity recovered was seen to be associated with

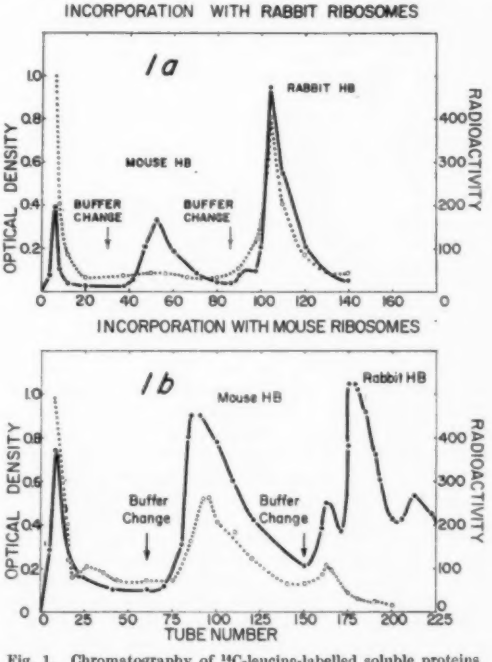

Fig. 1. Chromatography of ^{14}C-leucine-labelled soluble proteins using mouse and rabbit reticulocyte ribosomes. Column chromatography was performed as described in the text. Solid line, optical density at 415 mμ; dotted line, total radioactivity in counts per min. per tube

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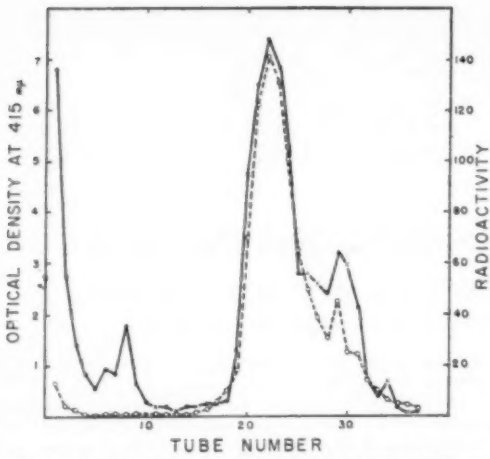

Fig. 2. Chromatography of soluble proteins after incubation of rabbit reticulocyte ribosomes with guinea pig liver ^{3}H-leucyl-ribonucleic acid. Dashed line, optical density at 415 mμ; solid line, total radioactivity in counts per min. per tube. Guinea pig liver soluble ribonucleic acid was labelled by incubation with ^{3}H-DL-leucine (150 mc./mmole) and 17 other unlabelled amino-acids, and isolated as previously described (ref. 8). Isolated ^{3}H-leucyl-ribonucleic acid (3·6 mgm.) was incubated at 37° with 18 mgm. of reticulocyte ribosomes and 42 mgm. of a reticulocyte enzyme fraction (ref. 9) plus $3·3 \times 10^{-4}$ *M* guanosine triphosphate, 0·07 *M* potassium chloride, $3·3 \times 10^{-3}$ *M* magnesium chloride, 0·02 *M* glutathione, and 0·033 *M tris* buffer, pH 7·5, in a volume of 9 ml. To increase the amount of soluble labelled protein, after 10 min. $3·3 \times 10^{-4}$ *M* adenosine triphosphate, 0·01 *M* creatine phosphate and 0·34 mgm. of creatine kinase were added and incubation was continued for another 30 min. Ribosomes were removed by centrifugation, and the proteins precipitated with ammonium sulphate and chromatographed as described (ref. 2)

rabbit hæmoglobin, and only 5 per cent with mouse hæmoglobin (Fig. 1*a*). On the other hand, when the reciprocal experiment was performed (mouse ribosomes incubated with the mixed supernatants) 52 per cent of the radioactivity recovered was associated with the mouse hæmoglobin and only 3 per cent with rabbit hæmoglobin (Fig. 1*b*). Thus, in these experiments, the hæmoglobin synthesized was the specific hæmoglobin of the species which contributed the ribosomes. The small amount of labelled protein in the other hæmoglobin is believed to be an artefact, since in a control experiment, when labelled rabbit hæmoglobin was mixed and chromatographed with unlabelled mouse hæmoglobin, 3–4 per cent of the recovered radioactivity was found in the mouse hæmoglobin peak, while 65 per cent was found in the rabbit hæmoglobin fraction. In each experiment radioactive protein was also found in a fraction which did not partition between resin and buffer, but passed directly through the column. It is not possible to conclude from these experiments that soluble components, such as soluble ribonucleic acid, have no function in sequence determination. For example, since soluble ribonucleic acid from both species was always present (and, besides, ribosomes cannot be completely freed from soluble ribonucleic acid[8]), it is possible that soluble factors contain sequence information, but that ribosomes can react only with soluble factors from the same species. However, the experiments do demonstrate that the reticulocyte cell-free system synthesizes a specific protein, and that the ribosome must play an important part in the determination of the amino-acid sequence of this protein.

In order to preclude the participation of endogenous soluble ribonucleic acid, experiments were performed with rabbit reticulocyte ribosomes using labelled amino-acyl-ribonucleic acid rather than free ^{14}C-amino-acid as starting material. Guinea pig liver ^{3}H-leucyl-ribonucleic acid was used since it differed in both organ and species from rabbit reticulocyte soluble ribonucleic acid. A large-scale incubation of the ^{3}H-leucyl-ribonucleic acid and reticulocyte ribosomes was required to provide sufficient labelled soluble protein for chromatography (Fig. 2). The results show that much of the radioactivity was recovered with the hæmoglobin fraction and the labelling of protein fractions using this guinea pig liver amino-acyl-ribonucleic acid was similar to that obtained with rabbit reticulocyte soluble ribonucleic acid. These experiments demonstrate the formation of labelled rabbit hæmoglobin from unrelated amino-acyl-ribonucleic acid, indicating that the determination of amino-acid sequence is controlled by the ribosome. In a similar manner, labelled hæmoglobin has been isolated after incubation of *E. coli* ^{14}C-amino-acyl-ribonucleic acid and reticulocyte ribosomes (von Ehrenstein, G., and Lipmann, F., private communication).

It was previously shown[1] that starting with free amino-acids labelled with carbon-14, the ratios of various amino-acids incorporated corresponded to the composition of crude globin. This was taken as evidence that the incorporation represented hæmoglobin synthesis. The pattern of incorporation of different amino-acids should be a function of the ribosome if amino-acid sequence is determined by the ribosome. Ratios of amino-acids incorporated starting with labelled guinea pig liver amino-acyl-ribonucleic acid were therefore studied. In each experiment, the ribonucleic acid was labelled with two amino-acids. One was ^{3}H-leucine (150 mc./mmole) and the other was either ^{14}C-valine or isoleucine or lysine (uniformly labelled, 6–10 mc./mmole). Rabbit reticulocyte ribosomes treated with deoxycholate were prepared as previously described[9]. Guinea pig liver ribosomes treated with deoxycholate were prepared in the same manner, but with twice the concentration of deoxycholate. The isolated labelled amino-acyl-ribonucleic acid was incubated in the standard way[10] with either 2·5 mgm. of reticulocyte or 3·7 mgm. of liver ribosomes for 3 min. The reaction mixtures were precipitated by the addition of 10 ml. of acid acetone (6 ml. of 4 *N* hydrochloric acid per litre of acetone) and the protein was washed as previously described[1,2]. The dried powders were each dissolved in 1 ml. of hyamine base and counted in the Packard 'Tricarb' liquid scintillation counter using a naphthalene, xylene and dioxane mixture[9]. The amounts of ^{14}C- and ^{3}H-amino-acids incorporated in each tube were calculated by the method of differential equations[11] using known mixtures of the labelled amino-acids as standards. The relative amounts of the amino-acids transferred to protein in the presence of rabbit reticulocyte ribosomes (Table 1) did not resemble the composition of the amino-acyl-ribonucleic acid, but that of rabbit globin. Very similar results were obtained when the incorporation of labelled free amino-acids was examined in the complete[9] reticulocyte cell-free system. The results with leucine and isoleucine were quite different for guinea pig liver and reticulocyte ribosomes, six times more isoleucine being incorporated into protein by the liver ribosomes. Thus, the amounts of different amino-acids incorporated appear to be controlled by the ribosome, as part of the selection needed to build a specific peptide chain.

Table 1. TRANSFER OF AMINO-ACIDS FROM GUINEA PIG LIVER AMINO-ACYL-RIBONUCLEIC ACID (RNA) TO RIBOSOMES

Amino-acid	Composition of amino-acyl-RNA	Transferred from amino-acyl-RNA G. pig liver ribosomes	Transferred from amino-acyl-RNA Reticulocyte ribosomes	Globin composition*	Incorporated from free amino-acids†
Leucine	100	100	100	100	100
Isoleucine	45	45	7	11	9
Valine	29	—	63	77	77
Lysine	63	—	71	67	—

Results are expressed as moles of each amino-acid per 100 moles of leucine.
* Microbiological assay of crude rabbit globin (ref. 12).
† 40 min. incubation in the complete cell-free system (refs. 1 and 3), all components from reticulocytes.

From this it might be thought that rabbit reticulocyte amino-acyl-ribonucleic acid and guinea pig liver amino-acyl-ribonucleic acid are completely equivalent in their action. That this is not so was demonstrated by the following experiment. ^{3}H-leucyl-ribonucleic acid was prepared from guinea pig liver as above. ^{14}C-leucyl ribonucleic acid was prepared from isolated reticulocyte soluble ribonucleic acid. To overcome the interference which might be caused by the different relative amounts of amino-acids carried by the two types of soluble ribonucleic acid, by differences in the extent of labelling, or by other unknown differences between the preparations, the two leucyl-ribonucleic acids were mixed and then incubated with guinea pig liver or rabbit reticulocyte ribosomes. 0·2 mgm. of guinea pig liver amino-acyl-ribonucleic acid (0·41 mμmoles ^{3}H-leucine) and 0·4 mgm. of rabbit reticulocyte amino-acyl-ribonucleic acid (0·42 mμmoles ^{14}C-leucine) were incubated for 3 min. at 37° with either 2·5 mgm. of reticulocyte deoxycholate-ribosomes or 3·7 mgm. of guinea pig liver deoxycholate-ribosomes under conditions previously described[10]. The incubation mixtures were washed up and counted in the liquid scintillation counter as described here. Table 2 presents the results of two experiments, showing that more leucine was transferred to guinea pig liver ribosomes from guinea pig liver amino-acyl-ribonucleic acid (^{3}H-leucine) than from reticulocyte ribonucleic acid (^{14}C-leucine) and vice versa. It appears that while no function was found for amino-acyl-ribonucleic acid compounds in determining the nature of the protein synthesized, some species differences may exist in soluble ribonucleic acid structure which result in a greater rate of transfer with ribosomes of the same species.

All the results given here rest on the conclusion that labelling of hæmoglobin represents a limited synthesis of this protein. The evidence for this point of view has been discussed[2,6]. However, the final test of the synthesis of a specific protein is the demonstration that the amino-acids are incorporated in their correct locations in the polypeptide chain. This has not yet been shown in any cell-free system. The first stages of such an investigation have provided further evidence supporting the conclusions given here. Three types of hæmoglobin, each labelled with radioactive leucine, were prepared and 'fingerprinted' as described below and the distribution of the radioactive leucine in various peptides determined.

Table 2. TRANSFER OF LEUCINE FROM MIXED AMINO-ACYL-RIBONUCLEIC ACID (RNA)

Source of ribosomes	Amount of transfer: Rabbit reticulocyte ^{14}C-leucyl-RNA mμmoles	Amount of transfer: G. pig liver ^{3}H-leucyl-RNA mμmoles	Ratio*
Rabbit reticulocytes	0·047	0·043	1·12
	0·049	0·041	1·21
Guinea pig liver	0·026	0·035	0·74
	0·024	0·034	0·71

* The ratio is the amount transferred from reticulocyte leucyl-RNA divided by that from liver leucyl-RNA.

^{3}H-leucyl-ribonucleic acid was prepared from guinea pig liver using ^{3}H-leucine at a specific activity of 3,000 mc./mmole and 1·2 mgm. of this isolated amino-acyl-ribonucleic acid was incubated with 7·5 mgm. of reticulocyte ribosomes for a total of 40 min. The hæmoglobin fraction was isolated by ammonium sulphate fractionation as described here. A 'uniformly labelled' hæmoglobin was prepared from 2 ml. of intact rabbit reticulocytes incubated with 2 mgm. of DL-leucine-1-^{14}C (specific activity 10·3 mc./mmole) for 6 hr. under conditions previously described[2]. Equal amounts of the two hæmoglobins were mixed and digested with trypsin[13]. 1·25 mgm. of the digest was applied to a sheet of Whatman No. 3 mm. filter paper and fingerprinted by the technique of Katz *et al.*[14] except that butanol : pyridine : water (4 : 3 : 3) was substituted as the chromatographic solvent. The paper was sprayed lightly with 0·025 per cent ninhydrin in ethanol and developed for 10 min. at 80° in a water-saturated atmosphere. The peptide spots were cut out and eluted[15] and the eluates were dried down in 20 ml. counting vials. The material was counted in the liquid scintillation counter as described here but with the omission of hyamine from the counting mixture. Under these conditions tritium and carbon were counted with efficiencies of 10 and 90 per cent, respectively. A third hæmoglobin fraction was prepared by incubation of reticulocyte ribosomes for 20 min. with free ^{3}H-leucine (3,000 mc./mmole) as described elsewhere[1,3] and treated in an identical manner. The assignment of peptides to alpha and beta chains was based on separation of these chains on '*CG*-50' columns in an acid–urea gradient[16].

Table 3 shows the relative amounts of labelled leucine found in the peptides of the alpha (valyl–leucyl) chain of rabbit hæmoglobin, and in several regions of the fingerprints which did not show ninhydrin staining. Similar results were noted in the beta-chain peptides. It is seen that the pattern of labelling in the three types of labelled material is similar. The peptides which carry labelled leucine after the incubation of intact reticulocytes also carry labelled leucine from the cell-free labelling experiments, while the peptides which were not labelled in the whole cell (presumably due to the absence of leucine) were not labelled in either cell-free system. In addition, a number of blanks which did not show ninhydrin colour were counted and showed a low level of activity. These results offer convincing evidence for the synthesis of hæmoglobin from heterologous amino-acyl-ribonucleic acid when rabbit reticulocyte ribosomes are used, and also for the validity of previous conclusions which assumed the synthesis of hæmoglobin in the cell-free system. For example, the validity of amino-acyl-ribonucleic acid as an intermediate in hæmoglobin synthesis is established. It should be noted that the exact percentages of radioactivity in the ^{14}C-labelled peptides (used as internal controls of recovery) vary between the two fingerprints, probably due to variations in elution from the paper.

The reticulocyte ribosome, rather than the transfer-ribonucleic acid, therefore, is responsible for the correct assembly of the hæmoglobin molecule. Which component of the ribosome carries the information remains to be determined. Treatment of ribosomes with tiny amounts of ribonuclease which did not release any detectable nucleotide material[3,17] resulted

Table 3. Radioactivity of Peptides of Alpha Chain of Rabbit Hæmoglobin

Peptide No.	Fingerprint 1† Intact cells ^{14}C-leucine	Fingerprint 1† Cell-free, free ^{3}H-leucine	Fingerprint 2† Intact cells ^{14}C-leucine	Fingerprint 2† Cell-free, ^{3}H-leucyl-RNA
1	2·5	4·6	9·2	8·7
2	8·2	5·1	3·8	1·9
3	31·1	12·5	32·2	36·9
4	7·2	6·6	6·1	6·8
5	18·2	20·4	20·3	20·4
6+7	1·3	2·0	1·8	1·1
8	3·2	2·3	1·4	1·6
9	0	0	0	0
10	16·4	19·8	13·5	13·0
12	12·2	26·5	11·6	9·7
13	0	0	0	0
14	0	0	0	0
Blank 1*	0	0	0	0
Blank 2	0	0	0	0
Blank 3	0	0	0	0

Radioactivity is expressed as the percentage of the total radioactivity in all peptides. 0 denotes less than 0·1 per cent.

† Fingerprint 1 is the mean of two experiments, fingerprint 2 the mean of three.

* 12 blanks taken in close proximity to the 12 ninhydrin-stained regions showed a total of less than 9 per cent of the recovered radioactivity in each case.

in a total loss of incorporating ability. Thus, it is likely that it is the ribonucleic acid of the ribosome which is important, as originally suggested by Crick[18]. Ribosomal ribonucleic acid, however, may consist of many components, some or all of which may have functions in protein synthesis unrelated to sequence determination. At the moment, a considerable amount of interest centres on a particular type of ribonucleic acid having a base composition related to that of deoxyribonucleic acid[19]. The work reported here makes it possible to examine directly the components, and the structural aspects of these components, which control the assembly of amino-acids in the correct sequence of the specific protein.

Note added in proof. Recent work on the 'messenger' ribonucleic acid of *E. coli* suggests that it is destroyed and replaced continuously in bacterial ribosomes during protein synthesis[20]. The species of hæmoglobin synthesized by rabbit reticulocyte ribosomes has been changed by incubation with soluble components from other species[21], under different experimental conditions from those used here. Thus, while specificity information may be added from soluble components, this was not essential for hæmoglobin synthesis in the experiments reported here. It is suggested that specificity information is therefore more stable in reticulocyte than in bacterial ribosomes, and that rapid 'turnover' of this information may not be an obligatory feature of protein synthesis.

This investigation was aided by grants to Prof. R. Schweet from the National Science Foundation and the National Institutes of Health, and to Dr. E. Russell from the National Institutes of Health.

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ANABOLIC AGENTS AND HISTAMINE METABOLISM

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TESTOSTERONE propionate has been shown to reduce urinary histamine output in rats[1,2,3]. Using new anabolic steroids with low androgenic activity, an attempt has been made to determine whether the androgenic or anabolic effect of testosterone is related to histamine metabolism. Various anabolic agents were compared with testosterone propionate in their effects on the excretion of free histamine, potassium, sodium and total nitrogen in rat urine.

Female albino rats weighing 140–220 gm. were individually placed in metabolism cages and fed 'Complan' diet (Glaxo Laboratories), a blend of dried skimmed milk, arachis oil, calcium caseinate, maltodextrin, cane sugar, essential minerals and vitamins.

During the examination of urinary nitrogen, potassium and sodium, food intake was kept constant by giving either 8 gm. 'Complan' or 8 gm. 'Complan' plus 1 gm. glucose daily depending on body-weight. 8 gm. 'Complan' provides 398 mgm. nitrogen, 2·2 mmole potassium, 1·58 mmole sodium and 36 calories. Tap water containing 0·013 mmole/l. of potassium and 0·04 mmole/l. of sodium was given *ad lib.*

For a test of androgenic activity of testosterone and 'Durabolin' (Organon), castrated male rats weighing 285–385 gm. were used and were fed normal laboratory rat pellets *ad lib.*

Urine was collected in acid (1·5 ml. 1 *N* hydrochloric acid in each collection vessel) over a 24-hr. period. Collection funnels and metabolism cages were sprinkled with distilled water (30–60 ml.). The combined volume of urine and washing fluid was measured and filtered through Whatman paper No. 1. Aliquots of filtrate representing 2- to 4-hr. samples were taken and neutralized. Final volume was made up with Tyrode solution to an appropriate concentration of histamine for assay.

Urinary free histamine was estimated by the biological assay method described elsewhere[2] and expressed in terms of the base.

Total nitrogen was determined by the micro-Kjeldahl method and sodium and potassium by the EEL flame photometer model *A*.

Table 1. Effect of 'Durabolin' and Testosterone Propionate on Urinary Histamine Output

Group	Av. body-wt. (gm.)	Dose (mgm.)	Average free histamine output/μgm./rat/day				Av. body-wt. gain (gm.)
			Control	Day after injection 4th	7th	11th	14th
Control	*(3) 163	—	32	36	43	44	20
'Durabolin'	(4) 180	1·25	31	13	2	3	38
Testosterone propionate	(3) 158	1·25	33	32	37	30	17

*No. of rats used.

Anabolic substances were all administered intramuscularly. Testosterone propionate ('Testoviron', Schering), 19-nor-androste-nolone phenylpropionate ('Durabolin', Organon) and 17α-methyl-17β-hydroxy-androsta-1, 4-diene-3-one ('Dianabol', Ciba) were used as steroid anabolic agents. The effect of growth hormone ('Somar-*A*', Armour) was also examined as an example of a different class of anabolic agents.

A preliminary examination showed that 'Durabolin', a powerful anabolic agent with low androgenic activity, reduced the output of free histamine (Table 1). These results indicated the existence of close relationship between anabolic mechanism and histamine metabolism. Therefore the effects of various anabolic agents on the excretion of free histamine were examined. The results are shown in Fig. 1.

'Durabolin' was the most potent substance in reducing urinary histamine output, and is followed by testosterone propionate. Although 1·25 mgm. testosterone propionate had no significant effect on the urinary histamine as shown earlier (Table 1), a larger dose (2·5 mgm.) produced a reduction (Fig. 1). Reduction of histamine output by 'Durabolin' lasted on the average for 40 days. 'Dianabol' was given in a dose of 1 mgm./rat/day for three consecutive days as it was not very effective in a single dose (1–2·5 mgm.). The effect of 'Dianabol' at this dose-level was similar to that produced by testosterone propionate (2·5 mgm.). Growth hormone was given to two normal females and to two ovariectomized females ; but since the effects observed were similar, both results were pooled to construct the figures shown in Figs. 1 and 2. Histamine excretion reached a minimum after the third injection and showed no further decrease after the fourth and the fifth injections. Neither continued injection for 7 days nor increased dose (2 mgm./day for 3 days) produced any further decrease. 'Heparin' (Leo) (2·5 mgm./day for 5 days) was also tested as it was reported to have an anabolic effect[3], but no significant effect was noted (Fig. 1).

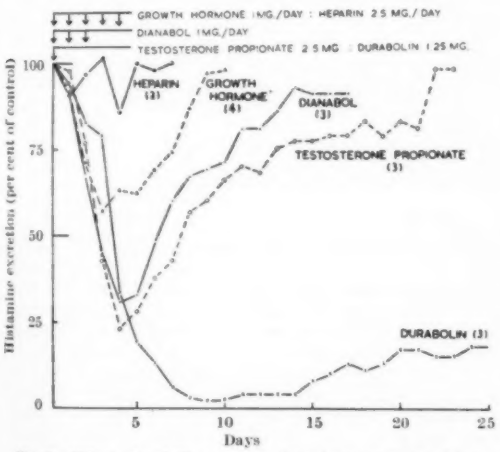

Fig. 1. Effect of anabolic agents on the urinary excretion of free histamine expressed as percentage of control-level (average value of daily histamine excretion during the last three days prior to the administration of drugs). The number in parentheses under the names of test substances indicates the numbe of rats used

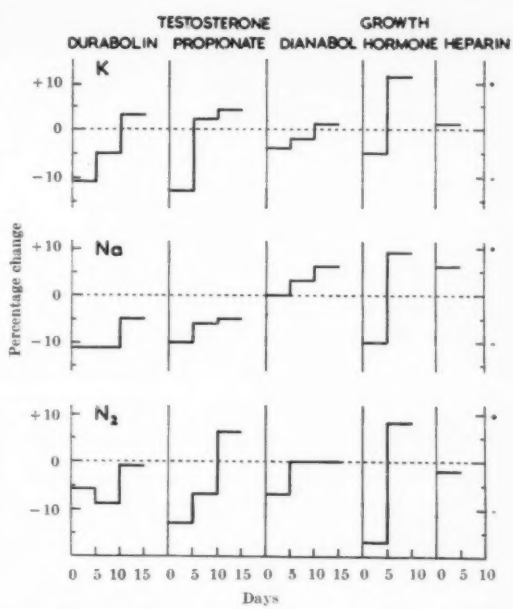

Fig. 2. Effect of anabolic agents on the excretion of urinary potassium, sodium and total nitrogen expressed as percentage change from the control-level. The investigation shown in Figs. 1 and 2 are concurrently carried out on the same rats

Anabolic substances are known to produce a retention of nitrogen, potassium and also sodium[4]. An attempt was therefore made during the investigation illustrated in Fig. 1 to examine whether the anabolic effect as reflected in the excretion of these substances is related to the potency in decreasing urinary histamine output. As shown in Fig. 2, 'Durabolin' showed a prolonged activity in retaining nitrogen, potassium and sodium, although testosterone propionate (2·5 mgm.) had a stronger effect during the first 5-day period. These effects were small with 'Dianabol'. Growth hormone produced a profound anabolic effect during its administration but showed rebound recovery over the control-level on discontinuation. 'Heparin' showed no significant effects indicating the absence of an anabolic effect.

Although the anabolic activity as reflected in the excretion of nitrogen, potassium and sodium appeared to be similar (Fig. 2), 'Durabolin' increased the body-weight more than testosterone propionate, as shown in Table 2. These rats, two in each group, were taken from the series illustrated in Fig. 1. This finding was further supported by the results shown in Table 1 although food intake was not controlled in this work. It should be noted that the absence of anabolic effect of testosterone propionate (1·25 mgm.) as reflected in gain in body-weight coincided with the absence of histamine reducing effect (Table 1).

Fifteen males, castrated 5 months previously, were divided into three groups—control, testosterone-treated and 'Durabolin'-treated. Testosterone- and

Table 2. Effect of 'Durabolin' and Testosterone Propionate on Gain in Body-weight

	Dose (mgm.)	Daily food intake 'Complan' (gm.)	Initial body-weight (gm.)	Wt. gain in 26 days after injection (gm.)
Control	—	8 8	160 165	15 10 Av. 12·5
'Durabolin'	1·25	8 8	165 177	30 21 Av. 25·5
Testosterone propionate	2·5	8 8	175 167	12 23 Av. 17·5

'Durabolin'-treated groups received two 1 mgm. doses of the respective drugs at an interval of 1 week, and the rats were killed one week after the last injection. As shown in Table 3, there was no appreciable effect on the weight of the kidneys; but significantly different effects were noted on the weight of the seminal vesicles. This result confirms the low androgenic effect of 'Durabolin' in comparison with testosterone propionate as reported by Overbeek and de Visser[6]. Therefore, it appears that the androgenic mechanism in the body is not related to histamine metabolism. Gain in body-weight was similar with 'Durabolin' and testosterone propionate.

In this work it is clearly shown that anabolic agents decrease output of urinary histamine. Evidence[2] has already been presented that testosterone propionate increases the capacity for histamine inactivation in rats. It is likely that 'Durabolin' and 'Dianabol' act by the same mechanism, the former more active and the latter slightly less active than testosterone propionate. An anabolic effect is believed to be produced by an increase in the activities of enzymes which synthesize protein. It is possible, therefore, that steroid anabolic agents may exert an effect on the output of urinary histamine through a potentiation of an enzyme system which inactivates histamine. Such an enzyme system might be common to both protein synthesis and histamine inactivation.

Table 3. Effect of 'Durabolin' and Testosterone Propionate on the Seminal Vesicles and the Kidneys of Castrated Male Rats

Group	Initial body-wt. (gm.)	Wt. gain in 2 weeks (gm.)	Seminal vesicle (mgm.)	Kidney wt. (gm.)
Control	314 ± 10*	−14 ± 6	53 ± 17	2·09 ± 0·12
'Durabolin'	356 ± 6	25 ± 1·6	100 ± 9	2·16 ± 0·09
Testosterone propionate	356 ± 11	24 ± 5·1	237 ± 44	1·94 ± 0·04

*Standard error.

The anabolic effect produced by growth hormone appears to be different from that produced by steroid anabolic agents[7–10]. The anabolic effect of growth hormone showed a different time-course from the steroids tested, and produced an effect on histamine excretion (Fig. 2). This may be taken as further evidence that growth hormone and steroid anabolic agents have different sites of action. Histamine output has been shown to be affected by testosterone propionate more readily in ovariectomized rats than in normal female rats[2]. No such hypersensitivity was noted with growth hormone in ovariectomized rats.

Although the effect of 'Dianabol' on the excretion of urinary total nitrogen was much smaller than that produced by testosterone (Fig. 2), it was shown by others[11] to produce a gain in body-weight equal to that produced by testosterone propionate. Both 'Dianabol' and 'Durabolin' have been shown to have very low androgenic activities at the dose-level used in this work[6,11] (Table 3). It is reasonable to conclude that it is not the androgenic but the anabolic activity of steroid compounds that is related to histamine metabolism.

Heparin in a dose of 2·5 mgm./rat/day for 5 days failed to produce an anabolic effect (Fig. 2). It also failed to reduce histamine excretion in the urine (Fig. 1). Granitsas[5] reported the anabolic activity of heparin in normal rats. The discrepancy between the results of Granitsas and this study is not clear at present and needs further investigation.

My thanks are due to Prof. R. A. Kenney for his kind assistance in the preparation of this manuscript. 'Durabolin' (Organon), 'Dianabol' (Ciba) and 'Testoviron' (Schering) were kindly provided by local agents of the respective firms.

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STAINING OF SULPHATED MUCOPOLYSACCHARIDES

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METHODS in use at present for detecting sulphated mucopolysaccharides consist of the exhibition of metachromasia with basic dyes, and a method using colloidal iron[1]. These methods tend to be rather unspecific, and nuclear staining tends to obscure the picture.

From the work of Stacey[2] it is apparent that sulphated mucopolysaccharides are found in mast cells (heparin), cartilage ground substance (chondroitin sulphuric acid) and some mucins. In addition, they have been isolated from other sites, for example, skin and cornea.

Previous work[3] has shown that nuclear fast red (Herzberg), when prepared as a 0·1 per cent stock solution in boiling 5 per cent aluminium sulphate solution which is diluted 1 to 2 with further 5 per cent aluminium sulphate solution for use, is a highly selective stain for mast cell granules, cartilage ground substance, and some mucins in tissue sections. This work has now been extended, the earlier conclusions having been confirmed.

Test slides of pure chemical substances dissolved in 5 per cent gelatin were prepared, fixed in formalin vapour overnight and stained 5–10 min. in the dye

solution. The substances used included the sulphated polysaccharides; heparin, from ox, sheep and pig, fucoidin[4], and a sulphated polyglucose, and the unsulphated polysaccharides, dextrin, dextran and starch. In addition, other known constituents of mast cells, namely, 5-hydroxytryptamine, histamine and 5-hydroxytryptophan, were used. Only the sulphated polysaccharides and heparin stained (Fig. 1*c*).

Tissues from members of all classes of vertebrates and sites, fixed in a variety of fixatives, both aqueous and alcoholic, were stained, and it was found that only those tissues which contained sulphated mucopolysaccharides reacted, for example, media or aorta, although mast cells when present in the tissues did stain. Only sections fixed in acetic Zenker failed to react in this way.

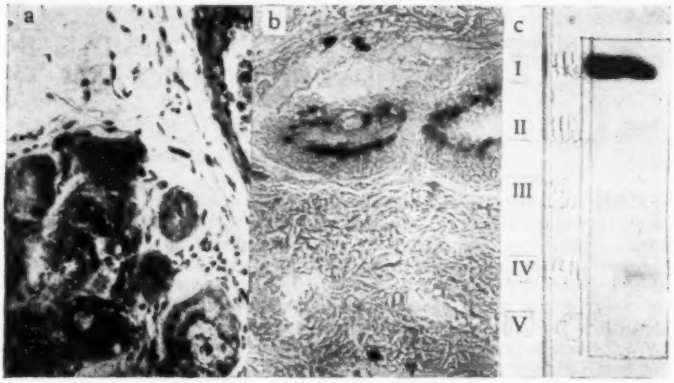
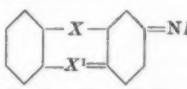


Fig. 1. (*a*) Ox trachea stained with 0·1 per cent aqueous methylene blue (×100). (*b*) An adjacent section stained with 0·1 per cent methylene blue in 5 per cent aluminium sulphate. Note only mucus and mast cells stain (×100). (*c*) Test slide of mast cell constituents in 5 per cent gelatin stained with 0·1 per cent nuclear fast red (Herzberg) in aluminium sulphate. I, heparin; II, histamine; III, serotonin; IV, 5-hydroxytryptophan; V, control gelatin blank

The stock solution of nuclear fast red in aluminium sulphate was buffered, using various buffers, to *p*H values between 0·5 and 7; at *p*H 7 a precipitate was formed. The *p*H did not affect the staining reaction.

These observations were extended to dyes of azo, quinone imine, phenyl methane, xanthine and phthalocyanine groups, in addition to several miscellaneous dyes, which were all prepared in the manner for nuclear fast red mentioned here. 0·1 per cent aqueous solutions were run in parallel as controls. In all, 56 different dyes were used, some of basic and some of acidic nature. The tissues used as test tissues were ox trachea and rat skin fixed in formol–dichromate (*p*H 6) and formol–calcium respectively. 6μ-sections were dewaxed, hydrated and stained for 5–30 min. in the dye solution, differentiated rapidly in 70 per cent alcohol, dehydrated in graded alcohols, cleared in xylene and mounted in polystyrene or Canada balsam.

With basic dyes prepared in aluminium sulphate solution, mast cell granules and cartilage ground substance stain deeply ortho- or metachromatically, depending on the dye used. Skin and mucin stain to a lesser extent and always orthochromatically (Fig. 1*b*). Those dyes which give the best results have the basic formula:



X = O, S, or N, X = C or N, R = CH_3 C_2H_5, etc.

The thiazines, toluidine blue and methylene blue, and the azines, neutral red and nuclear fast red, give the best results. Certain dyes give less specific reactions, staining, in addition, red blood cells, nuclei and all mucines. This is particularly noticeable with the rhodamines, but less with the phenyl methanes and dyes having large substituent groups attached to the benzene rings in the above formula, namely, cresyl fast violet, janus green. Triphenylmethane dyes tend to differentiate out rapidly and for that reason are not as satisfactory as the azines and thiazines. Basic dyes in aqueous solutions stain all the elements mentioned here, but nuclei and the cytoplasm of mucous cells stain too (Fig. 1*a*). Acidic dyes in aluminium sulphate solution stain all tissue elements deeply, including cartilage ground substance, but in aqueous solution the cartilage ground substance remains unstained, although the cell nuclei in the lacunæ stain.

In an attempt to find out what groups, if any, were specific for this reaction, sections containing sulphated mucopolysaccharides were methylated[5] and stained as mentioned. This prevented staining. Methylation has been reported to block various groups in tissue sections[5], including sulphate groups, and this seemed a possible explanation of its effect. This explanation was supported by the finding that sections containing unsulphated mucopolysaccharides, for example, umbilical cord, after sulphation[6] stained a uniform deep colour. It appears, therefore, that the sulphate groups are, in part at least, responsible for the reaction.

Equimolar concentrations of other aluminium salts, either as pure aluminium salts or as alums, were found to confer similar specificity of action on basic dyes, as did a number of salts of other metals. It appears from this that it is the cation which is responsible for the specificity of action, and by using a variety of different compounds it became apparent that only those cations which freely form co-ordination complexes confer specificity on the basic dyes.

It appears, therefore, that by preparing certain basic dyes in aluminium sulphate a highly specific stain for the detection of sulphated mucopolysaccharides in tissue sections is produced. The dye complex produced is probably a co-ordination compound of a suitable metallic cation and the dye, the reaction between the tissues and the complex being similar to mordanting in the textile dye industry.

I wish to thank Evans Medical Ltd., Bengers, Ltd., and Dr. D. M. Shepherd of the Pharmacology Department, Queen's College, Dundee, for supplying the heparins, dextran and sulphated polyglucose respectively.

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AN UNREACTIVE TYROSINE RESIDUE IN INSULIN AND THE EXCLUSIVE IODINATION OF THE *A* CHAIN

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It has been known for some time that it is possible to introduce sufficient iodine into insulin to bring about disubstitution of all the tyrosine residues present[1]. More recently it has been observed that the *A* chain is preferentially iodinated, particularly at lower levels of iodination[2]. The present article describes iodination conditions under which one tyrosine residue of the *B* chain appears to be entirely unreactive and another sufficiently unreactive to facilitate exclusive iodination of the *A* chain.

Iodination of proteins, including insulin, has generally been carried out between *p*H 5 and 10 (refs. 3 and 5). Although there are reports of iodination under more acidic conditions[3,4], no detailed study has been described using insulin. It was found that iodination of insulin by the McFarlane method[5] proceeds almost as well at *p*H 1 as at higher *p*H values, providing only partial iodination is attempted (Fig. 1). Even with larger amounts of iodine no more than about 6 atoms of iodine/mole could be taken up in the first 5 min. of the reaction.

Examination of the distribution of iodine–131 was carried out after disulphide splitting by Swan's method[7], followed by high-voltage paper electrophoresis[8] and autoradiography (Figs. 2 and 3). The figures show that it is possible to iodinate the *A* chain almost exclusively at the 1 atom/mole level at *p*H 1 but not at *p*H 9 (Figs. 2*e* and 3*f*). When more iodine was used, the proportion found in the *B* chain gradually increased (Figs. 2*a–d* and 3*b-e*). If iodination is first carried out at *p*H 1 to the 6 atoms/mole level with inactive iodine and afterwards at *p*H 9 with 2 atoms labelled iodine/mole, then 75 per cent of the radioactivity is associated with the *B* chain (Fig. 3*a*).

The uptake of 6 atoms of iodine could lead to the mono-substitution of the 4 tyrosine and 2 histidine residues, the di-substitution of only 3 tyrosine residues, or to di-substitution of some residues and mono-substitution of others. It is evident from the tyrosine and histidine distribution[9] that mono-substitution of all residues would result in an eventual predominance of iodine in the *B* chain. However, scanning for radioactivity in the appropriate regions (Fig. 3*b* and *c*) shows that this is not the case at *p*H 1. Also it is unlikely that histidine is involved since it is known to react much less readily with iodine than does tyrosine under a variety of conditions[10]. Analysis of enzymic digests of the split chains of insulin confirmed the presence of diiodotyrosine. Although monoiodotyrosine was also found, it is uncertain whether it represents a genuine product of iodination. Some of it is almost certainly formed by partial deiodination of the diiodo compound during proteolysis[11] and possibly also during the disulphide splitting reaction.

On the basis of the above it is concluded that *p*H 1 iodination at high iodine-levels probably results mainly in the di-substitution of 3 tyrosine residues, leaving one inaccessible at least for the first 5 min. of the reaction. Another tyrosine group is also iodinated less readily than the other two residues at low iodine-levels. Both these less reactive tyrosine residues are apparently located in the *B* chain.

Iodination of insulin at *p*H 1 brings about opalescence and eventually flocculation as more and more iodine is added. This could be interpreted as resulting from aggregation similar to that observed during formation of fibrous insulin[12]. One site of attach-

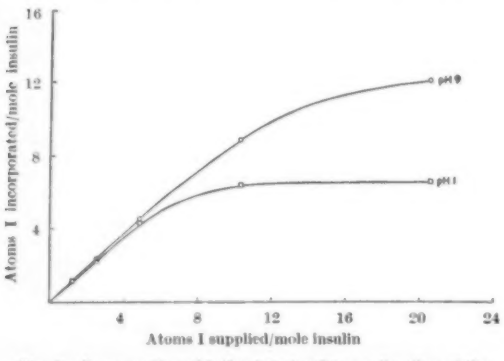

Fig. 1. Incorporation of iodine into insulin as a function of the amount of iodine supplied. Insulin (2 mgm. B.D.H. crystalline) was dissolved in 0·5 ml. 0·1 *N* hydrochloric acid. The following were added: 0·5 ml. buffer (containing per 100 ml.: □, *p*H 1–10 ml. 1 *N* hydrochloric acid + 3 ml. 2 *M* glycine + 12 ml. 2 *M* sodium chloride; ○, *p*H 9–1·3 ml. 2 *N* sodium hydroxide + 9 ml. 2 *M* glycine + 12 ml. 2 *M* sodium chloride). 0·5 ml. 0·1 *N* sodium hydroxide (in *p*H 9 experiments only), water to give a final volume of 4·5 ml. after iodine monochloride addition and iodine monochloride (3·3 μatoms I/ml.)[5] containing between 5 and 50μc. iodine-131 depending on requirements. After 5 min. reaction, 0·5 ml. 100 per cent trichloroacetic acid was added and the protein precipitated. The supernatant was withdrawn and the precipitate washed with 5 ml. 10 per cent trichloroacetic acid. Suitable aliquots of supernatants were counted. (Atoms I incorporated/mole insulin = atoms I supplied/mole insulin $\times \left(\frac{\text{added activity} - \text{supernatant activity}}{\text{added activity}}\right)$

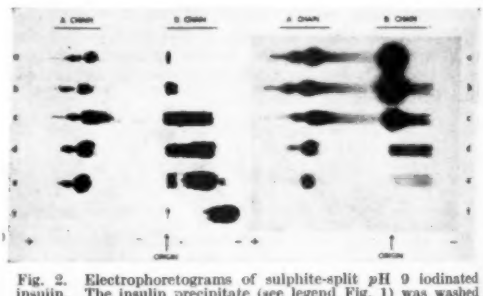

Fig. 2. Electrophoretograms of sulphite-split *p*H 9 iodinated insulin. The insulin precipitate (see legend Fig. 1) was washed with ether to remove trichloroacetic acid, dissolved in Swan's reagent[7], the mixed chains precipitated after the –SS– splitting and the *A* and *B* chains separated by paper electrophoresis (3,800 V., 2·5 hr.) in pyridine : acetic acid : urea as described by Dixon and Wardlaw (ref. 8). After drying and removal of excess urea, the paper was autoradiographed (right) and then sprayed with diazotized sulphanilic acid (Pauly) reagent (left). Amounts of iodine supplied and incorporated (expressed in atoms/mole) are as follows: (*a*) 20·6 and 12·1, (*b*) 10·3 and 8·9, (*c*) 4·8 and 4·5, (*d*) 2·5 and 2·3, (*e*) 1·2 and 1·1. Electrophoretogram (*f*) contains untreated insulin only. Minor components probably represent deamidated products. Because of the higher radioactive content, relatively less of the mixed chains was applied to spots (*a*) and (*b*), which accounts for the apparent reduction in the amount of Pauly-positive material

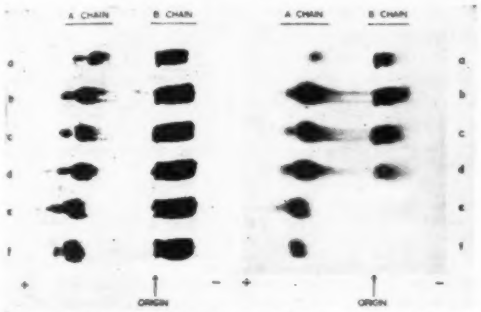

Fig. 3. Electrophoretograms of sulphite-split *p*H 1 iodinated insulin. The insulin was treated as described in the legend of Fig 2. Insulin in electrophoretogram (*a*) was first iodinated at *p*H 1 by addition of 20·6 atoms inactive I/mole and then at *p*H 9 by addition of 2·5 atoms I (containing iodine-131)/mole. At *p*H 9 2·3 atoms I/mole were shown to be incorporated by radioassay; at *p*H 1 presumably 6·6 atoms I/mole were introduced. Amounts of iodine supplied and incorporated (expressed in atoms/mole are as follows: (*b*) 20·6 and 6·6, (*c*) 10·3 and 6·4, (*d*) 4·8 and 4·3, (*e*) 2·5 and 2·2, (*f*) 1·2 and 1·1. Left: After spraying with Pauly's reagent. Right: Autoradiograph

ment during association of insulin monomers may be in the vicinity of those portions of the *B* chains which contain the masked tyrosine residue. In this connexion it is of interest that Porter[13] gave a similar explanation for the observed masking of one of the *B* chain histidine residues.

Although earlier work seemed to indicate that all tyrosine groups of insulin were equally accessible[14,15], more recent work indicates that both tyrosyl residues in the *B* chain are hydrogen bonded[16] and that one tyrosyl group may be more strongly bonded than the other[17] within the 6.000 molecular weight unit. Since iodinations were carried out under *p*H conditions in which internal hydrogen bonding may no longer be taking place[16,18], the hypothesis involving inter- rather than intra-molecular factors is favoured.

Investigation of the masked tyrosine in the *B* chain is being directed towards establishing which of the tyrosyl residues is involved. Distribution of the iodine within the preferentially iodinated *A* chain is also being examined.

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RELATIONSHIP BETWEEN VELOCITY OF CARDIAC CONTRACTION AND SIZE OF THE HEART: A HYPOTHESIS

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A. V. HILL[1] suggested that "cardiac muscle may be able to change its intrinsic speed according to conditions . . . the heart, unlike the skeletal muscle, becomes intrinsically quicker when the frequency of its beat rises". Recently, experiments on cardiac muscle strips and papillary muscle have provided support for this suggestion[2,3]. These findings imply in regard to regulation of performance of the intact heart that cardiac muscle, unlike skeletal muscle, has no fixed length. This fact, considered with the experiments mentioned and the results of recent work on the effect of calcium ions infused into the intact heart of a dog[4], has suggested the following hypothesis.

For a given cardiac load (stroke volume and peripheral resistance), there is a relationship between the velocity of myocardial muscle fibre shortening and the momentary initial length of fibre or initial volume of the intact heart. Thus, an increased velocity of shortening of cardiac muscle fibres is associated with an increased extent of shortening and so leads to a decrease in the end-systolic volume of that beat and a decrease in the end-diastolic volume of the subsequent beat.

The cardiac contraction has been characterized as analogous to a skeletal muscle twitch[5]. Two separate loads for a given contraction must be considered: (1) the initial load, during the isovolumic phase of contraction, as defined by the end-diastolic volume; (2) the 'ejection' or after-load which operates during the auxotonic phase, and is defined by the volume of blood discharged and the aortic pressure during ejection, that is, the pressure-time curve between diastolic aortic pressure and end-systolic pressure. The cardiac contraction is a unique after-loaded performance since the after-load tends to disappear during ejection. This is due to the fact that tension of the wall in a hollow organ decreases proportionally to the cube root of the volume ejected, so that volume shrinkage may more than counteract rise in cavity pressure.

According to the working hypothesis, the final or contracted length, the end-systolic volume, is only a partial function of the initial length, the end-diastolic volume. The extent of shortening of cardiac muscle

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must also be a function of the velocity of shortening for a given load, and the velocity of shortening can be related to the active state, which has been shown to be a discrete period in a muscle twitch. In the case of the heart, the active state would be partitioned between its two phases of contraction. Thus, if the initial load is achieved earlier in the contraction, the potential for further shortening is increased. At the same time, the ejection-after-load is decreased in the ensuing contraction. Thus, the potential for shortening in the heart is greater than would otherwise occur in an ordinary or simple after-loaded contraction.

Skeletal muscle contractile response to a load, the force of contraction, is customarily considered relative to the fixed or *in situ* length, since it has been shown that the maximum force or tension is attained at that length. On the other hand, end-diastolic cardiac volume, the equivalent of initial length of the myocardial fibres, may change from moment to moment in the same individual. There is really no fixed initial length of fibre in the heart. For example, it has been shown that an appreciable decrease in volume of the heart occurs when an individual shifts to the erect from the sitting or prone position[6].

The experiments of Abbott *et al.*[2] and of Sonnenblick and McCallum[3] have established that the velocity of contraction of cardiac muscle for a given after-load may be augmented: (1) by increasing the heart-rate; (2) by increasing the initial length of the cardiac fibres; (3) by the application of positive inotropic agents such as the catechol amines and calcium. The results have been interpreted as representing an increase in the intensity of the active state, since the increased velocity of shortening for a given load was shown to occur despite absence of change or even a decrease in the duration of the active state[2].

In relation to the present hypothesis, one of several factors leading to an increase in shortening velocity in the cardiac muscle strip will lead, in the intact heart, to: (1) an earlier attainment of the aortic diastolic pressure-level; (2) an increased velocity of shortening in relation to the ejection after-load; (3) an increased amount of ejection and systolic muscle shortening, that is, a smaller end-systolic volume, and (4) a smaller end-diastolic volume in the subsequent beat.

Several findings may be cited as evidence for this hypothesis. Wiggers and Katz[7], in the open-chested animal, showed that increasing the aortic resistance at a constant stroke volume resulted in a transient augmentation in initial volume, followed by a subsequent decrease. Sarnoff *et al.*[8], using an *in vitro* preparation operated at constant coronary flow and stroke volume, confirmed this observation and termed it the 'homometric' regulation of the heart to distinguish it from the ordinary 'heterometric' mode. In Sarnoff's preparation the initial length of fibre, as measured with a strain gauge, increased at first when aortic resistance is augmented at fixed stroke input and then decreased. Consistent with the hypothesis presented, these findings are amenable to the interpretation that an increase in initial length of cardiac muscle will initiate an increase in the velocity of its contraction which, at constant stroke input, will result in a decrease in size of heart.

Infusion of catecholamines was shown by Wiggers and Katz[7] to result in a decrease in size of the heart. Recently[4] we found that calcium infusion into the open-chested anæsthetized dog heart operated at controlled cardiac input led to: (1) an increase in the rate of ventricular development of pressure; (2) a decrease in end-systolic volume of the heart. Table 1 shows the results.

Table 1. Effect of Infusion of Calcium Ions on the Heart of the Dog*

	ΔP/ΔT† mm./sec.	Stroke volume c.c./min.	ΔCircumference‡ per cent of initial
Control	433	7·8	
Calcium ions infused§	622	7·0	−21

* In the open-chested anæsthetized animal, the heart operated at constant cardiac input. (Mean of three animals.)
† Rate of rise in pressure of left ventricle.
‡ Change in left ventricular end-diastolic circumference as measured with a mercury-in-rubber gauge (ref. 10).
§ Continuous infusion of 10 per cent calcium gluconate.

Finally, Rushmer *et al.*[9] has found consistently that exercise in the intact unanæsthetized animal is associated with a relatively constant stroke volume and stroke work and that the increased cardiac output in exercise is due to an increase in heart-rate. Associated with these changes is a decrease in size of the heart. In one experiment it was shown that, if the response of heart-rate to exercise is tape-recorded and used to pace the heart in a resting unanæsthetized animal, a decrease in size of the heart ensued[9]. These findings would fit the hypothesis presented if they were interpreted to imply an increased velocity of shortening.

The cause of an increase in velocity of cardiac contraction in Rushmer's experiments might be due to an increase in heart-rate or an outpouring of catecholamines, or both. There has, as yet, been no unequivocal experimental demonstration, free of filling changes or of changing inotropic influences, that an increase in heart-rate will increase contraction velocity and decrease size of the heart.

The significance of an equilibrium between velocity of contraction, extent of shortening and the volume of the heart is far-reaching and provides an important supplement to the Starling mechanism for cardiac control. Thus, an increase in initial length, heart-rate, and the advent of positive inotropic influences are postulated as an effective counterbalance to factors that require greater initial length to sustain an augmented load. Those factors which initiate stretch of cardiac fibres are still not clearly understood. They may include: (1) metabolic factors, since cardiac work at smaller initial volume is less efficient; (2) augmented input (diastolic) loads; (3) negative inotropic factors. Finally, apparent or real changes in contractility and/or distensibility may be involved, both in increasing and decreasing the velocity of contraction and the extent of shortening.

I thank Dr. L. N. Katz for helping me.

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ISOLATION AND PROPERTIES OF DEOXYRIBONUCLEIC ACID FROM MAMMALIAN SPERM

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MANY of the properties of the deoxyribonucleic acids (DNA) from numerous sources are well documented, yet relatively little is known of the DNA from mammalian spermatozoa despite their central role in the transmission of genetic determinants. Although DNA can be isolated easily from other mammalian cells, and from the sperm of fish and lower vertebrates, such isolation from mammalian spermatozoa has hitherto been extremely difficult, if not impossible[1]. This has probably stemmed from the inaccessibility of the DNA towards rigorous chemical and physical manipulations which appears due largely to the nearly impervious keratin-like membrane surrounding the sperm-head. The DNA of ram and boar spermatozoa, for example, remains insoluble and bound to a residual protein even after contact for 30 min. with 1·0 *N* sodium hydroxide[2]. Similarly the DNA of bovine sperm is inaccessible to extraction with 0·1 *N* sodium hydroxide[3].

In a brief report in 1950[4], a DNA isolated from defatted human sperm by a procedure involving extensive grinding followed by tryptic digestion was described. The physical properties of the product, however, suggested that considerable degradation had accompanied the isolation. When we applied this procedure to bull sperm it failed to yield DNA. The finding of a sulphur-rich, proteolytic enzyme-resistant keratin-like residue in ram and bull spermatozoa (refs. 5 and 6*a*, *b*) and their increased solubility in thioglycollate[2] suggested that prior rupture of protein-sulphur linkages might lead to an appropriate procedure for the isolation of DNA.

Accordingly, the effect of exposure of fresh mammalian sperm to three groups of agents which affect sulphur linkages was studied. These included oxidizing, mercaptide-forming and reducing agents. Use of only the reducing agents permitted the isolation of the DNA. Pretreatment of sperm with 2-mercaptoethanol, thioglycollic acid and British Anti-Lewisite (2,3-dimercaptopropane) at *p*H 7 was effective at a concentration of 0·25 *M*, and cysteine and sodium sulphite were less so. However, this treatment alone was not sufficient, and DNA was released into solution only after subsequent incubation with trypsin. Reversal of the sequence was ineffective.

Several samples of semen from healthy, highly fertile bulls and pooled samples from rabbits were obtained from the New York Animal Breeders' Corp., Ithaca, New York, through the kind co-operation of Dr. H. O. Dunn and Dr. R. H. Foote, of the Department of Animal Husbandry, Cornell University. Sperm-counts and motility-rates were determined

Table 1. Properties of DNA from Mammalian Sperm

	Bull 1	Bull 2	Bull 3	Bull 4	Rabbit	Human
Atomic N/P ratio	3·45	3·37	3·85	3·53	3·77	3·46
$S_{20,w,0}$	18·1	23·4	19·3	19·8	12·8	16·5
$M_s \times 10^{-6}$	4·5	8·2	4·9	5·4	1·8	4·0
(η), dl./gm.	49	53	45	47	19	40
$M\eta \times 10^{-6}$	5·0	5·7	5·2	5·1	2·3	4·4
T_m, °C.	90·0	87·0	88·0	87·0	88·0	89·0
A abs., %	37	36	33	35	33	30

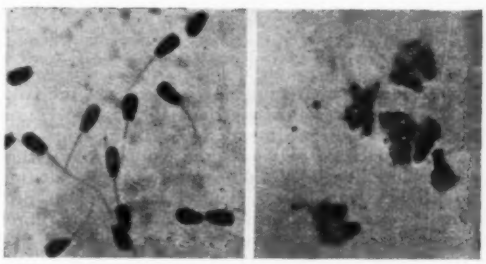

Fig. 1. (left), Bull spermatozoa before treatment. Unstained, unfixed (× 500); (right), bull spermatozoa after exposure to 0·25 *M* β-mercaptoethanol for 2 hr. Unstained, unfixed (× 500)

prior to shipment of the freshly frozen specimens. Usually, 2–3 ejaculates from individual bulls (12–16 ml., about $1{\cdot}5 \times 10^9$ cells per ml.) were pooled.

We are indebted to Dr. J. MacLeod, of the Department of Anatomy of Cornell University, for several specimens of semen from normal, healthy medical students. The specimens were processed within ½ hr. of collection. Various methods of homogenization (Potter–Elvehjem, Virtis at 14,000 r.p.m., Mickel's vibrator) and attempts to rupture the sperm cell by freeze-thawing, treatment with deoxycholate or detergents, as well as extraction with phenol or strong salts, failed to release DNA from bull spermatozoa. Similarly, treatment with the enzymes lipase, chymotrypsin, trypsin and lysozyme, or exposure to 9 *M* urea, also failed to release the DNA.

A sperm pellet was obtained by centrifuging the semen for 20–30 min. at 8,000*g* in a refrigerated centrifuge in order to remove the seminal plasma, which is known to contain nucleases[7]. The sperm pellet was washed twice with 0·15 *M* sodium chloride –0·015 *M* sodium citrate and once with ethanol, followed by a 1 : 1 mixture of alcohol–ether to remove the lipoprotein from the capsule[8]. The washings were removed by centrifugation at 8,000*g* for 30 min. To about 100 mgm. of the lipid-free sample were added 20·0 ml. of 0·15 *M* sodium chloride –0·015 *M* sodium citrate containing 2 per cent 2-mercaptoethanol (0·25 *M*) and the mixture incubated for 2 hr. with gentle magnetic stirring in the cold. The effect of this treatment on the morphology of the spermatozoa is shown in Fig. 1. Trypsin (10 mgm.) was then added and the incubation continued for 1 hr. at room temperature. The sample was then centrifuged at 12,000*g* for 1 hr. The supernatant was kept, and the sediment was re-suspended in 10 ml. of saline-citrate containing 5 mgm. of trypsin and incubated for 1 hr. at room temperature and centrifuged. This enzyme step was usually repeated once more, to assure complete release of DNA. To the supernatants obtained after tryptic digestion were added 2 volumes of ethanol, and the nucleic acid fibres which precipitated were removed with a glass rod and redissolved in 0·01 *M* sodium chloride with gentle stirring in the cold room.

XUM

Table 2. Base Composition of DNA from Mammalian Spermatozoa

Sperm	Hydrolysis	Bases in moles per cent				A/T	G/C	T/C	Pur/Pyr	$\frac{A+T}{G+C}$
		Adenine	Thymine	Guanine	Cytosine*					
Bull	Formic	28·7	28·2	21·6	21·6	1·02	1·00	1·30	1·02	1·31
Bull	$HClO_4$	28·9	26·3	22·8	22·1	1·09	1·03	1·19	1·06	1·23
Bull	HCl	25·9	29·4	22·9	21·7	0·99	1·05	1·35	0·96	1·24
Bull†	Formic	28·8	27·5	22·3	22·0	1·05	1·07	1·31	1·06	1·30
Human	Formic	30·5	28·9	19·9	20·6	1·05	0·96	1·40	1·01	1·47
Human‡	Formic	29·8	32·5	18·6	19·3	0·92	0·96	1·70	0·94	1·64

* Includes 5-methylcytosine.
† Data from Wyatt, G. R. (ref. 27), recalculated in moles per cent.
‡ Data from Chargaff, E., Zamenhof, S., and Green, C. (ref. 30); base composition is average of two determinations, recalculated in moles per cent.

After the first treatment with trypsin, the preparation was at times so viscous that efficient separation of DNA from the sediment by centrifugation was difficult. In those instances the sample was either further diluted with the saline-citrate or if separation was still not complete the entire mixture was precipitated with 2 volumes of ethanol. The fibres which formed were removed with a glass rod and redissolved in saline-citrate to which a fresh aliquot of enzyme had been added.

Further deproteinization was carried out by the procedure of Kay, Simmons and Dounce[9]. To the 0·01 *M* sodium chloride solution of the DNA, 0·1 volume of 4 per cent 'Duponol' (sodium dodecyl sulphate) in 45 per cent ethanol was added and the mixture stirred at 4° C. for 3 hr. The salt concentration was then raised to 1 *M*, and the stirring continued for 45 min. The mixture was centrifuged for one hour at 13,000*g*, and the DNA in the supernatant precipitated with two volumes of ethanol. In order to reduce the content of protein to less than 0·5 per cent, the level detectable by the method of Ramachandran[10], the preparation was subjected to four or five additional treatments with 'Duponol'. This procedure permits the isolation of about 96–99 per cent of the DNA present in the sperm. This was determined by extracting the remaining tissue sediments after the isolation of DNA with hot trichloracetic acid according to Schneider[11]. These extracts contained less than 4 per cent of the total DNA. DNA, determined by the Burton modification[12] of the Dische reaction, constitutes about 15 per cent of the alcohol–ether-extracted spermatozoa studied.

Phosphorus determinations were carried out acccording to King[13] and the Nessler reagent was used for nitrogen analysis. The atomic nitrogen to phosphorus ratios (N/P, Table 1) are well within the range found for mammalian DNA. Viscosities were determined at various concentrations between 15 and 70 μgm./ml. in 0·2 *M* sodium chloride with a capillary viscometer[14] at shear-rates of 158, 101 and 48 sec.$^{-1}$. Intrinsic viscosities were obtained by extrapolation to zero concentration and zero shear. Solutions containing 0·002 per cent DNA in 0·2 *M* sodium chloride were analysed at 59,780 r.p.m. (259,700*g*) (refs. 15 and 16) in a 'Spinco' model *E* ultracentrifuge equipped with ultra-violet optics, and average sedimentation coefficients, $S_{20,w}$, corrected to 20° C. and water were calculated[17]. With the exception of the DNA from rabbit sperm, which from the sedimentation and intrinsic viscosity values suggest a molecular weight considerably lower than those of the other specimens, the preparations from bull and human sperm showed intrinsic viscosities (η) between 40 and 50 dl./gm. and sedimentation coefficients of about 19–22 *S*. These values are consistent with average molecular weights (Ms and $M\eta$) of about $4–5 \times 10^6$ (ref. 18). Buoyant densities were determined by the method of Meselson, Stahl and Vinograd[19] in a density gradient formed by centrifugation (44,770 r.p.m.) of 8 *M* cæsium chloride solutions to which 2 μgm. of sperm DNA and 1·0 μgm. of a standard DNA–^{15}N from *Pseudomonas aeruginosa* with a density of 1·742 (kindly supplied by Dr. J. Marmur) were added. The buoyant density found for the DNA from bull and human spermatozoa was 1·701, which is about the same as that of DNA from other mammalian tissues[20].

A special heating device attached to the Beckman *DU* spectrophotometer permitted determination of temperature-absorbancy curves according to Marmur and Doty[21]. The midpoint (T_m) of the rise of absorbancy at 260 mμ as a function of temperature averaged about 88° C., and the hyperchromic effect due to temperature, $\Delta_{abs.}$, averaged about 35 per cent. These data indicate a high degree of nativeness. This was supported by the fact that the preparations failed to react with formaldehyde; this is consistent with the behaviour of nucleic acid in the undenatured (double-stranded) form[22]. This is further supported by application of the procedure of Zamenhof *et al.*[23], in which transforming DNA is added to a tissue prior to extraction. When pneumococcal DNA active in transformation to streptomycin resistance was added to bull sperm, and the DNA was isolated by the procedure outlined here, the product still retained its transforming activity.

The values of T_m and buoyant density are consistent with a guanine plus cytosine content of about 43 per cent[20,21]. Base analyses were obtained by hydrolysis with 88 per cent formic acid[24], with 72 per cent perchloric acid[25] and with 6 *N* hydrochloric acid[26]. Wyatt's isopropanol-HCl solvent system[27] was used for paper chromatography. The ultra-violet-absorbing spots were eluted with 0·1 *N* hydrochloric acid and the concentrations of the individual bases determined with the Beckman *DU* spectrophotometer (Table 2)[28].

We feel that the base analyses of the DNA hydrolysed by formic acid are the most reliable. The low

Table 3. Chromatographic Fractionation of Bull Sperm DNA*

Chromatographic region	Composition of eluant Molarity, NaCl	Molarity, NH_3	% of original	$S_{20,w}$
Original			100	20·8
1	0–0·5		41	17·7
2	2·0	0·1	16	20·0
3	2·0	0·1–1·0	10	
4	2·0	2·0	25	22·4
5	(0·5 *M* NaOH)		7	

* Bull sperm DNA (2·5 mgm. in 5·5 ml. of 0·01 *M* phosphate, *p*H 7·0) was applied to a 0·8 × 7·2 cm. column of 'Ecteola'-*SF*–1 and fractionated as described previously (ref. 31). Total recovery of DNA was 96 per cent. The fractions obtained in region 3 consisted of a low, ill-defined band. $S_{20,w}$ was determined on samples taken from the peak tubes in the regions indicated.

Table 4. AMINO-ACIDS RELEASED FROM BULL SPERM DNA*

Aspartic acid	Threonine	Serine	Glutamic acid	Alanine	Valine	Methionine	*Isoleucine*	Leucine	Lysine	Histidine
11·2	8·2	26·0	13·2	6·1	12·4	3·7	2·0	4·4	9·9	2·7

* Determined on a 27 mgm. sample in an automatic amino-acid analyser (ref. 33) after hydrolysis by refluxing with 6 *N* HCl for 24 hr. The glycine was not determined since purines break down under these conditions to give appreciable amounts of glycine.

The results are expressed as moles of amino-acids/100 moles of all amino-acids actually found. Phenylalanine and tyrosine were present as traces. Neither cysteine nor cystine was detected. Together, these amino-acids amount to about 0·1 per cent of the mass of the DNA sample. We are grateful to Dr. M. E. Balis for carrying out the analysis.

thymine value following perchloric acid hydrolysis and the low adenine after hydrochloric acid hydrolysis are consistent with the experience of others who have reported losses of these bases by these procedures[26,29]. The thymine to cytosine ratio (T/C) previously reported[29] for human sperm DNA is considerably higher than the present values (Table 2). The ratios of purines to pyrimidines (Pur/Pyr) are close to 1·0 as found for other mammalian tissues[1].

The DNA from bull spermatozoa was fractionated on a column of the anion exchanger 'Ecteola' using an elution system which yielded DNA in 5 chromatographic regions[31]. The sedimentation coefficients of the DNA in the peak tubes in each region were determined. The results, given in Table 3, show that bull sperm DNA, like that of calf thymus[32], consists of fractions which vary in molecular size and which can be separated on an 'Ecteola' column.

The requirement of the reducing agent used in the isolation of DNA from mammalian sperm suggests the presence of a special sulphur-containing linkage between the DNA (or a component attached thereto) in the sperm nucleus and the protein coat cleavage of which is necessary before the nucleic acid can be released. It is less likely that the protein coat consists of a highly cross-linked, rigid, disulphide-containing protein which merely engulfs the DNA, since release of the DNA should attend the disintegration of the coat, which is brought about by grinding, homogenization, etc.

Accordingly, the DNA isolated from bull sperm was hydrolysed with 6 *N* hydrochloric acid and examined for the presence of amino-acids[33]. The results (Table 4) indicate the presence of protein (or amino-acid) to the extent of about 0·1 per cent, with a remarkably high serine content. The relatively low content of basic amino-acids, the presence of many other amino-acids, and the absence of cystine have also been noted in highly purified DNA from human white cells, spleen and placenta[34]. It is noteworthy that the only sulphur-containing amino-acid detected was methionine, and this was present in small amounts (3·7 per cent). It is possible that sulphur-containing amino-acids may have been removed by the tryptic digestion step. The significance of the amino-acid content is being investigated since this might hold a clue to an understanding of the action of β-mercaptoethanol, which is needed for eventual release of the DNA. This pre-treatment is not required for release of DNA from the sperm of lower forms such as fish, invertebrates and birds, and such differences may therefore be a reflexion of evolutionary development.

These results with sperm DNA are of particular interest since the source material is not only devoid of RNA but consists, presumably, of a homogeneous cell population in contrast to the usual heterogeneous cell-types found in tissues. In addition, sperm is the only reasonably available mammalian haploid cell. Yet, obvious differences with the DNA of diploid tissues are not revealed by the properties given here.

The amino-acids (or protein) which are present in these as well as in so many other preparations described in the literature and which resist rigorous efforts to remove them may be co-valently linked to the DNA. Although these are present to only a small extent, they may play an influential part in the transmission and determination of heritable characteristics and in differentiation. Hence, a critical examination of the amino-acid composition of the DNA and a comparison of preparations from different tissues and sources may reveal an unsuspected basis of the biological activity of these polymers.

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SURFEN: A QUINOLINE WITH ONCOGENIC AND HEPARIN-NEUTRALIZING PROPERTIES

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SURFEN (*bis* - 2 - methyl - 4 - amino - chinolyl - 6 - carbamid) has been described as an oral heparin antagonist more potent than protamine sulphate. Experiments were undertaken to ascertain its degree of inhibitory power on heparin *in vivo* and to determine its toxicity. Earlier personal communications have discounted untoward effects following oral administration to rats for 90 days (Dr. Jose Steinhardt, personal communication).

Eighteen mature Swiss mice were fed 'Purina' chow infiltrated with surfen. These animals were divided into groups 1 to 4, receiving a minimum intake of 60, 120, 150 and 300 mgm./kgm./day of surfen, respectively. Groups 1, 3, and 4 consisted of 3 animals each, while group 2 contained 9 animals. Groups 1, 3, and 4 received the diet for 80 days, after which time all animals were autopsied; those in group 2 were fed surfen for only 60 days. All animals were observed daily for evidence of toxic effects. Three untreated animals were autopsied as anatomical controls. The whole mouse colony served as physiological controls. Micro-coagulation times were performed on 3 groups of 2 rabbits given oral surfen before and after heparinization. Two untreated heparinized rabbits were used as coagulation controls.

Mice subjected to prolonged surfen ingestion at dose-levels slightly in excess of those thought to be of therapeutic value developed lymphosarcomata and lesions suggestive of nutritional deficiency (Table 1).

Table 1. Microscopic Changes observed in Control and Surfen-treated Mice

Microscopic observations	Group 1	Group 2	Group 3	Group 4	Control
Surfen mgm./kgm./day	60	120	150	300	0
Animals/group	3	9	3	3	3
Lymphosarcoma, per cent animals with tumour	0	33	100	0	0
Per cent animals with lymphocytic infiltrates	67*	33†	0	0	0
Gastric mucosal hyperplasia					
Total thickness	450μ	500μ	500μ	300μ	400μ
Epithelial thickness	24μ	30μ	25μ	2μ	20μ
Polarity of nuclei	absent	absent	absent	absent	present
Pseudostratification	present	present	present	present	absent
Depot fat; lipocyte size	20μ	15μ	10μ	12μ	20μ
Lipid-containing cells/HPF	100	20	20	30	100
Distorted, giant hepatic cells/HPF	1	1	3	5	1
Colloid casts, distal tubules/LPF	1	0	1	0	0

* Two animals showed lymphocytic infiltrations of soft tissue and viscera similar to those seen in incipient mouse leukæmia or severe, generalized viral diseases. Although a virus would have affected the control and group 4 animals with equal probability, the absence of unequivocal sarcoma in group 1 necessitates interpretation as benign.

† Florid lymphocytic infiltrates were encountered in one additional animal. Prominent lymphocytic aggregates were evident in others. Absence of tumour masses necessitated that these cases be classified as 'lymphocytic infiltrates' rather than 'lymphosarcoma'.

The lymphosarcoma in one animal in group 3 became apparent after 55 days on the diet and accounted for the death of the animal on day 71. A second animal in this group showed the tumour at day 70 and the third sarcoma was discovered at autopsy. Those sarcomas in group 2 developed after day 80.

Evidence of dietary deficiency became apparent after only 10 days on surfen. Most marked gross lesions were seen in group 4 about 50 days through the experiment. At this time, the extremities, noses, and tails of all animals became eczematoid and œdematous. Two animals lost portions of their tails following necrosis of distal segments. Trauma was not a factor since physical activity was virtually nil at this time. These lesions partially regressed near the end of the experiment. Hepatic changes consisted of marked pleiomorphism of size and shape of parenchymal cells. Some disruption of lobular architecture was seen, but no necrosis was apparent to account for this. Nuclei were pycnotic and cytoplasm was deplete of glycogen. Severity of dietary deficiency closely paralleled dosage of surfen. Terminal coagulation times were normal.

Rabbits given 300 mgm./kgm. of surfen within 10 min. before, and at any time after, heparinization showed no change in coagulation times when compared with control heparinized animals. However, the feeding of surfen 4–6 hr. prior to heparinization resulted in a moderate depression of coagulation

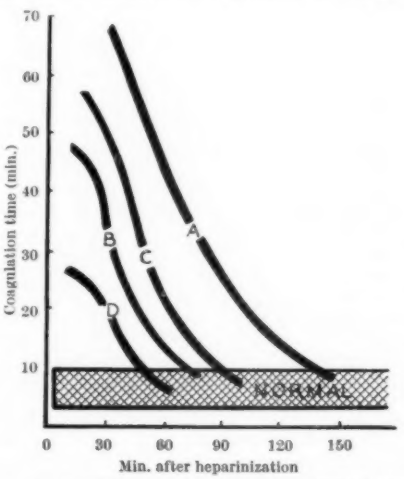

Fig. 1. *A*, Animals receiving 2 mgm./kgm./heparin intravenously. Curves of animals administered oral surfen 10 min. prior to, or at any time after, heparinization were identical with animals receiving no surfen. *B*, same as *A* except for heparinizing dose of 0·5 mgm./kgm. *C*, Animals receiving 2 mgm./kgm. heparin but pretreated with 300 mgm./kgm. surfen 4 to 6 hr. beforehand. *D*, Same as *C* except for heparinizing dose of 0·5 mgm./kgm.

times to about 50 per cent of that seen in controls (Fig. 1).

Although the series is small, presence of lymphosarcoma in a third of the animals in group 2 and all animals in group 3 strongly indicates that surfen is oncogenic at these dose-levels. Based on a spontaneous incidence of one per cent for group 2, p is calculated as 0·000080 and for group 3 as 0·000001.

Absence of sarcomata in group 4 may be explained by the suppressing effect oncogenic substances exert on neoplasia in high doses[4], and by the resistance of malnourished and/or vitamin-deficient animals to tumour development[5]. The findings, in 2 animals of group 1, and 3 animals of group 2, suggest that a prolonged test-period would result in increased tumour induction even at the low dosage in group 1. The oncogenic property of surfen is supported by its hyperplastic effect on gastro-intestinal mucosa. Tumours have not been observed before in this colony of Swiss mice. Although we accepted the manufacturer's chemical description of surfen, we did subject the substance to fractionation in an attempt to disclose oncogenic impurities. The product was homogeneous. Environmental factors would have probably induced neoplasia with equal frequency in the control colony and in group 1. The quinolines have not previously been incriminated in oncogenesis, except in the styryl complex[2], and indeed have displayed oncolytic properties[5].

The other changes observed indicate that surfen interferes with food and/or vitamin utilization. Similar changes are seen in vitamin-deficient animals but not in starved animals[1]. Although the diet was not enriched with vitamins, food intake was sufficient in all groups to maintain health in untreated animals. The liver pathology was suggestive neither of starvation nor of hepato-toxic effect.

Coagulation work performed using heparinized rabbits pre-treated with surfen indicated moderate heparin-neutralizing effects. Surfen is apparently slowly and imperfectly absorbed in the digestive system, and shows activity only if given several hours in advance of heparinization. In this respect, it is inferior to protamine sulphate.

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NERVE ENDINGS IN THE MOLE'S SNOUT

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CONCENTRATED in a small area of hairless skin at the tip of the snout of the near-blind mole are numerous cutaneous nerve endings. In all probability they play an important part in facilitating path-finding and feeding while the animal is either on or below the surface of the ground.

During waking hours the snout is constantly used in a probing fashion (accompanied by head movements) to explore the immediate environment, although the general direction of the animal's movements towards food or away from danger is presumably plotted beforehand in response to olfactory and vibratory sensations. A memory system must also be involved as even when traversing unaccustomed territory the animal may only deviate momentarily from its set course in order to circumvent local obstacles. Observations during life confirm that the snout region is markedly pink in colour and that it is also freely mobile. They also suggest that it is semi-erectile in character since during periods of hyperactivity or stress it assumes a redder hue and becomes more prominent (for example, as during obligatory swimming, when the animal progresses at a great speed and holds its snout well above the water-level). After death, the snout becomes paler and diminishes in size. However, it is not known whether such local circulatory changes could affect the character of responses from nearby nerve endings sensitive to external physical stimuli[7].

At present light-microscope work using *Talpa europaea* has confirmed the classical work of Eimer[4] that there are, in the snout region, many profusely innervated epidermal papillæ passing into the dermis (the so-called 'Eimer's organs'). The presence of encapsulated nerve endings near the deep aspect of Eimer's organs has been recorded by Ranvier[10], Bielschowsky[1] and Boeke[3]. More recently Cauna and Alberti[3] have denied the presence of encapsulated

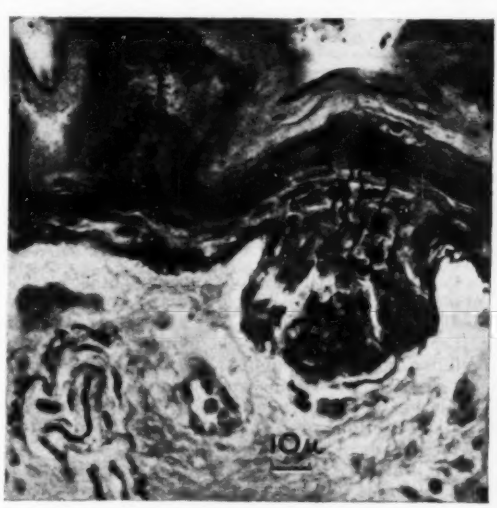
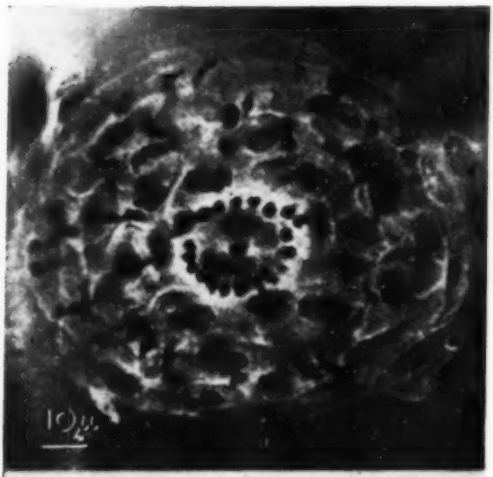


Fig. 1. Vertical section through the skin of the tip of the mole's snout. An Eimer's organ lies to the right. Part of the dermal plexus supplying it and some of the nerve 'terminals' within it can be seen. An encapsulated nerve ending of an unnamed variety lies to the left and intervening between these two structures is a blood sinus. (This material, together with that shown in Fig. 2, was stained by a modified Cajal method)



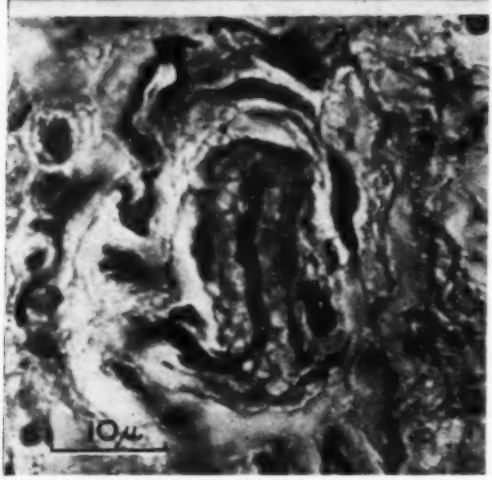


Fig. 2. Above, transverse section through an 'Eimer's organ' showing the axial nerve terminal closely related to paired cells of an inner core. Satellite nerve terminals are arranged circumferentially in a clear annular space. Lying peripherally are the epidermal cells that constitute the bulk of Eimer's organ.

Below, longitudinal section (high power) through an encapsulated nerve ending of an unnamed variety showing a single central axon surrounded by a comparatively simple core and an ill-defined capsule

nerve endings in this region, and have suggested, by implication, that the Eimer's organs are themselves multi-purpose transducers. The present work, together with unpublished electronmicroscopic findings (Quilliam), clearly demonstrate the presence of encapsulated nerve endings of an unnamed variety in the dermis and have considerably amplified the original descriptions and hand-drawn illustrations of them published 35–90 years ago[1,2,10].

The importance of the present findings lies in the fact that it is now no longer necessary to attach an extraordinary functional significance to the Eimer's organs, and in particular, to ascribe to them the unique ability of subserving a comprehensive spectrum of cutaneous sensory modalities.

Fig. 1 shows a vertical section through the skin of the tip of the mole's snout. Above can be seen the undulating profile of the epidermal surface and towards the right is an Eimer's organ in longitudinal section. A few of the fibres of a dermal nerve plexus supplying it from below are visible and a number of ascending nerve 'terminals' (or possibly 'pre-terminals') can be seen traversing it vertically on their way towards the more superficial layers of the epidermis. Located below and to the extreme left is an encapsulated nerve ending the long axis of which in the photomicrograph illustrated happens to be orientated vertically. It is separated from the Eimer's organ previously identified on the right by a blood sinus.

Fig. 2 (above) depicts a typical Eimer's organ in transverse section. A single nerve terminal, axially, is clearly seen to be surrounded by an array of circumferentially disposed satellite nerve terminals. The latter are situated in a clear annular zone that separates the outer core of epithelial cells from the paired cells of the central core which themselves closely invest the axial terminal. The unusually orderly pattern of these grouped nerve terminals may well form a satisfactory structural basis for the fine tactile discrimination that the mole practises during normal activity. Unfortunately, it has not yet proved possible to differentiate histologically between axial and satellite nerve terminals except in respect of diameter measurements and of their relative positions within an Eimer's organ. Consequently, it is not known whether any of the satellite terminals are collaterals of the dermal fibre which is in continuity with the axial terminal. In view of the many bundles of myelinated fibres present in the dermis it would appear likely that every Eimer's organ is innervated by several myelinated fibres. The relatively large number of satellite terminals in an Eimer's organ further suggests that at least some of the dermal fibres supplying it must branch prior to entry into its deep surface—presumably in a subjacent nerve plexus.

Fig. 2 (below) is an enlargement of the encapsulated nerve ending shown in Fig. 1. The capsule is rather poorly differentiated, and the core tissue surrounding the nerve terminal is somewhat scanty. However, nuclei can be made out in the latter on either side of the terminal (at 4 o'clock and 9 o'clock) and several small granules can also be distinguished (at 12 o'clock). The single nerve terminal is almost straight once it has entered the core and ends without visible structural modification. Since some of its morphological features suggest that it belongs to an unnamed type of ending not adequately described hitherto its fine structure will be described elsewhere (Quilliam).

The cellular organization of these encapsulated endings exhibits both certain similarities to and a number of divergences from the pattern typical of the Pacinian corpuscle in the cat. However, it may be tentatively assumed that, functionally, the former behaves like the latter in responding to high-frequency pressure changes[5,6,8].

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LETTERS TO THE EDITORS

RADIOASTRONOMY

Spectrum of Galactic Radio Emission

THE general radio emission from the Galaxy has now been accounted for as the synchrotron radiation of high-energy electrons in a magnetic field. An unsatisfactory aspect of this explanation is the relation between the observed radio spectral index α, where radio flux is proportional to (wave-length)$^{\alpha}$, and the expected energy spectrum of the cosmic-ray electrons, the number density of which should also vary exponentially according to (energy)$^{-(1+S)}$. In these circumstances the indices are related by $\alpha = S/2$.

It has been shown by Tunmer[1] that if the electrons are supposed to be generated by the collision of cosmic-ray protons with atomic nuclei, the value of S will depend on the predominating mechanism by which the electrons lose energy. Assuming a proton number density proportional to (energy)$^{-2.5}$, the following values of S will be expected for the various loss mechanisms:

Synchrotron radiation	$S = 2{\cdot}5$
Bremsstrahlung radiation	$S = 1{\cdot}5$
Ionization losses	$S = 0{\cdot}5$

The measured value of $\alpha = 0{\cdot}37 \pm 0{\cdot}04$ for the frequency-range 38–178 Mc./s. (ref. 2) corresponds with a value $S = 0{\cdot}74 \pm 0{\cdot}08$. This value appears to indicate that losses are primarily governed by ionization; unfortunately, Tunmer's paper indicates that the only important loss for the electrons concerned with radiation in this frequency-range is the synchrotron radiation itself. Radiation at 100 Mc./s. is produced by electrons with energy of the order of 10^9 eV. in a field of 10^{-6} gauss. The actual conditions of emission cannot be far different from these, and it seems inescapable that a value of S near 2·5 should be found, corresponding to $\alpha = 1{\cdot}25$.

If the electrons are not formed throughout the energy spectrum, but only at higher energies than those concerned, afterwards falling through the range by synchrotron losses, then a radio spectral index of 0·5 would be found. Even making this extreme hypothesis of the life-history of the electrons, the expected value of α is still greater than that observed.

It is natural to suspect the experimental evidence, and indeed this is now being checked as rigorously as possible. However, some new observations at 408 Mc./s., soon to be published by Pauliny-Toth, support the low value of radio spectral index. The experiment reported by Molozzi, Franklin and Tyas[3], in which galactic radio noise at 3·8 Mc./s. was measured by a satellite-borne radio receiver, also lends some support. Here the measured field-strength E can be related to an effective black-body temperature T by:

$$T = \frac{3\lambda^2}{8\pi kB}\,\frac{E^2}{Z_0}\,\varepsilon^{1/2} \quad \text{(Hugill, in preparation)}$$

For the northern hemisphere, Molozzi's results give $E \simeq 0{\cdot}6$ μV./m., and it seems unlikely that this result is much affected by the ionosphere. Hence $T \simeq 1{\cdot}3 \times 10^6$ °K., while the value to be expected by extrapolating Costain's results from 38 Mc./s. to 3·8 Mc./s. using $\alpha = 0{\cdot}37$ is $2{\cdot}8 \times 10^6$ °K. The indication here is of an even lower value of α at the lower frequencies.

No theory has yet attempted to resolve this problem; the purpose of this communication is merely to point out a serious discrepancy between theory and experiment, which is not apparent from the papers by Tunmer[1] and Hoyle[4]. Further examination of the processes of loss and gain of energy by the electrons of cosmic-ray energies seems to be required.

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ASTROPHYSICS

Production of Neon in Stars

WE have recently made certain measurements of the properties of some levels in neon-20 which are related to the production of neon in stars. In particular we have shown that the 4·97-MeV. level has odd parity and $J = 2$. Consequently neon-20 cannot be formed through this level by thermonuclear reactions between helium and oxygen-16 as has been suggested to explain its production in stellar interiors after the hydrogen has been consumed[1,2]. The suggestion arose from the close proximity of the 4·97-MeV. level in neon-20 to the energy of oxygen-16 + α as shown in Fig. 1. For the level to be effective the parity would have to be $(-)^J$ where J is the total angular momentum.

The first evidence that the level has $J\pi = 2-$ arose from γ–γ correlations in the neon-20 ($pp'\gamma\gamma$) reaction that established the total angular momentum as 2 (ref. 3) and the unexpectedly long life-time of $1{\cdot}9^{+3.5}_{-1.0} \times 10^{-12}$ sec. found by the Doppler shift technique in our experiments[4] using the carbon-12(^{12}C αγ)neon-20 reaction. The assignment of negative parity has now been established by our investigations (to be published) of neon-20($pp'\gamma$)neon-20. In this reaction measurements of the linear polarization[5,6] of the 3·34-MeV. γ-ray by Compton scattering combined with the angular distribution of the γ-ray defined its electric or magnetic character and thereby demonstrated that the levels concerned at 4·97 MeV. and 1·63 MeV. have opposite parity. The parity of the 1·63-MeV. level is positive (ref. 7 and A. E. Litherland and E. Almqvist, D. A. Bromley, J. A. Kuehner and B. Whalen, to be published). A parity of $(-)^{J+1}$ for the 4·97-MeV. level has since been deduced (A. E. L., to be published) from measurements (E. Almqvist, D. A. Bromley, J. A. Kuehner and B. Whalen, to be published) of the α-particle angular distributions in the carbon-12(^{12}C α)neon-20 reaction, and $J\pi = 2-$ has been confirmed by α–γ correlations in this same reaction (H. E. G., A. E. L. and M. A. C., to be published).

The next available level for helium thermonuclear reactions is at 5·63-MeV. This can be formed by the α + oxygen-16 reaction (H. E. G., A. E. L. and

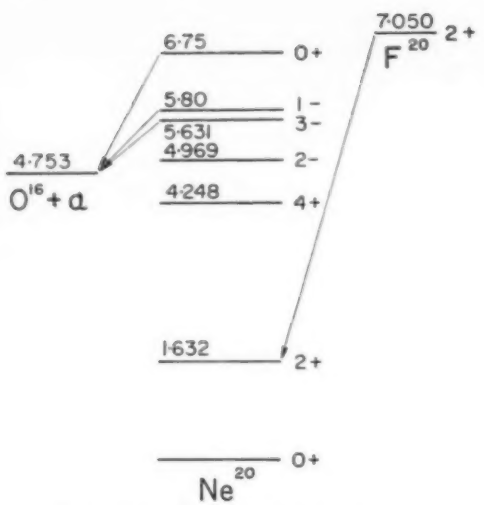

Fig. 1. Diagram for low-lying levels in neon-20

M. A. C. and J. A. Kuehner and E. Almqvist, to be published) and preliminary experiments (A. E. L. and H. E. G., unpublished) on the reaction at an α-particle energy corresponding to this level yield a value of $(2J+1)\frac{\Gamma_a\Gamma_\gamma}{\Gamma} = 0{\cdot}003 \pm 0{\cdot}002$ eV. The 5·63-MeV. level in neon has now been shown to have $J\pi = 3-$ (J. A. Kuehner, to be published) and $\Gamma_\gamma/\Gamma = 0{\cdot}07 \pm 0{\cdot}01$ (H. E. G., A. E. L. and M. A. C. and J. A. Kuehner and E. Almqvist, to be published). Cameron (private communication) has recently estimated that, under these conditions, neon-20 could be formed through the 5·63-MeV. level at temperatures above 5×10^8 °K. in supernova explosions. He has also pointed out the possibility that the strong neon lines observed in certain stars may be neon-22 formed in the reactions nitrogen-14(αγ)fluorine-18($\beta^+\gamma$) oxygen-18(αγ)neon-22.

The level at 5·80 MeV., which was first discovered in measurements of the sodium-23(pα)neon-20 reaction[8], has been shown to have $J\pi = 1-$ (J. A. Kuehner, to be published) and $\Gamma_\gamma/\Gamma = 0{\cdot}003 \pm 0{\cdot}003$ (J. A. Kuehner and E. Almqvist, to be published). It would be of great interest to measure $(2J+1)\frac{\Gamma_a\Gamma_\gamma}{\Gamma}$ for this level in the oxygen-16(αγ)neon-20 reaction, since it may also contribute to neon formation in stars.

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SEISMOLOGY

Possible Method for increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave

In seismology a method has been developed of examining the focal mechanisms of earthquakes by studying the azimuthal variation of the first movements on seismograms[1]. In extending this method to differentiating between small earthquakes and explosions a basic experimental problem is the detection of the initial movement when its amplitude is of the order of only 10^{-7} cm. in the frequency-range 1–10 c.p.s., and is therefore comparable with the background microseismic noise-level at the quietest sites. The purpose of this communication is to suggest and illustrate a possible technique of increasing the signal : noise ratio.

It is generally considered that the majority of seismic noise consists of surface waves, a characteristic of which is that their amplitude decreases approximately exponentially with depth[2]. The first arrivals at a recording site at distance between 200 and 1,000 km. from a shallow event are P_n waves which have been refracted along the Mohorovičić discontinuity and emerge at the surface with an angle of incidence of about 50°. Allowing for surface reflexion their amplitude varies with depth by a factor of two. Thus, accepting the postulate that the noise is predominantly surface waves, it is immediately obvious that an increase in signal : noise ratio can be obtained by placing the detector deep undergound, typically a factor of 10 being gained at 3 c.p.s. for a depth of 4,000 ft. In this communication we are suggesting that a further improvement of signal : noise ratio can be obtained through the use of two detectors, one at the surface and the other at a depth of, say, a wave-length at the peak recording frequency. The signal at the surface detector is then regarded mainly as noise, and is subjected to a frequency dependent attenuation, higher frequencies being attenuated most, to give the noise signal theoretically expected at the lower detector. The two signals are now subtracted so that the noise is eliminated, and the required *P*-wave signal, slightly modified, remains.

To test the method, model seismic studies have been carried out. The model consists of a 1/16-in. sheet of 'Perspex' 48 in. × 12 in. (Fig. 1). The transmitter at the surface, T_1, generates a burst of 'white' noise, about 750 μsec. in duration, while the 'buried' source, T_2, transmits a 10-μsec. pulse. Two detectors are placed two-thirds of the way along the model, D_1 at the surface and D_2 at a depth of 1 in., corresponding to about one wave-length for a surface

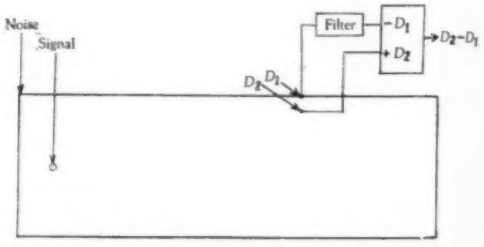

Fig. 1. Diagram of arrangement of transmitters and detectors on model

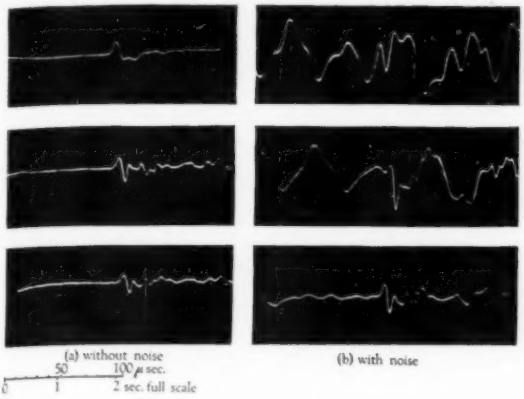

Fig. 2

wave with a frequency of 50 kc./sec. which is the dominant frequency of the P-wave. The timing of the two transmitted signals is such that the P-wave from T_2 arrives at the detectors about half-way through the burst of surface waves from T_1. The signal from D_1 is passed through a filter having a frequency attenuation closely resembling the theoretical surface wave attenuation at the depth of D_2, and this is then subtracted from the signal from D_2 at the appropriate relative gain. (To allow for frequency-dependent phase-changes in the filter, which are approximately linear with frequency, the two detectors are staggered horizontally by about $\frac{3}{4}$ in.) Typical records are shown in Fig. 2, the gain in signal : noise ratio being readily apparent.

The basic principles behind the method were developed in private discussions with Prof. J. Oliver of Columbia University and we are pleased to acknowledge his help.

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PHYSICS

Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with Barium Oxide

It has been known for some years that irradiation with ultra-violet light can have an important effect on the photoelectric and thermionic properties of a cathode coated with barium oxide. Ultra-violet irradiation at room temperature causes an enhancement of photoelectric emission induced by illumination of the cathode by light of other wave-lengths[1,2], and a pronounced change in the spectral sensitivity curves for photoelectric emission[1–5] and photoconductivity[5] is observed after ultra-violet treatment. The enhancement is only observed at low temperatures and may be removed by heating the cathode to about 700° K. Ultra-violet irradiation may also cause an enhancement of thermionic emission[2]. It is the purpose of this communication to report the results of preliminary observations of the effect of ultra-violet irradiation on the dark electrical conductivity of cathodes coated with barium oxide, as distinct from its enhancing effect on photoconductivity[5].

The experimental tubes employed contained a tubular 'O'-nickel cathode base, provided with an insulated tungsten heater, and coated over a portion of its length with barium oxide. A tungsten thermocouple wire was welded internally to the nickel tube, allowing the measurement of cathode temperature. Embedded in the cathode coating was a helical probe of fine nickel wire. A cylindrical nickel anode with a small circular window pierced in it was mounted coaxially with the cathode. A side-arm attached to the glass envelope of the experimental tube bore a 'Pyrex'-to-quartz graded seal with a plane quartz window at the quartz end, aligned so that the cathode could be illuminated through the quartz window and the window in the anode. The barium oxide coating was prepared by the usual method of pyrolysis of a sprayed barium carbonate suspension. The tubes were submitted to the usual schedules of breakdown, outgassing and activation by drawing thermionic emission current, and after sealing off from the vacuum system an active barium getter was fired.

Measurement of the electrical conductance of the cathode sample could be made by applying small potentials (up to 100 mV.) between the probe and cathode base and measuring the conductivity current on a sensitive galvanometer. The conductivity current-voltage characteristic at any desired cathode temperature T °K. was traced and the conductance σ of the barium oxide sample at that temperature could be obtained by taking the gradient of the characteristic at zero applied potential. The curvature of the characteristic was slight, and only a small error was incurred in later stages of the work when, for the sake of speed of observation, the difference in galvanometer readings for $\pm$ 20 mV. applied between the probe and cathode base was taken as a measure of the conductance.

In determining the effect of ultra-violet irradiation on dark conductivity, the variation of conductance with temperature for the cathode under investigation was first determined before any ultra-violet treatment was applied. The conductivity of well-activated cathodes was sufficiently high to allow measurements to be commenced at a cathode temperature of about 800 °K. and continued down to room temperature. The results are best displayed by plotting $\log \sigma$ versus $1/T$, as shown in Fig. 1*a*. The conductivity plot shows the well-known Loosjes–Vink[6] pattern in which the two linear portions are interpreted as representing a conduction process in which free electron flight across the pores of the oxide coating predominates at high temperatures, and a process of bulk or surface semiconduction through or over the surface of the oxide crystallites operates at low temperatures. In the

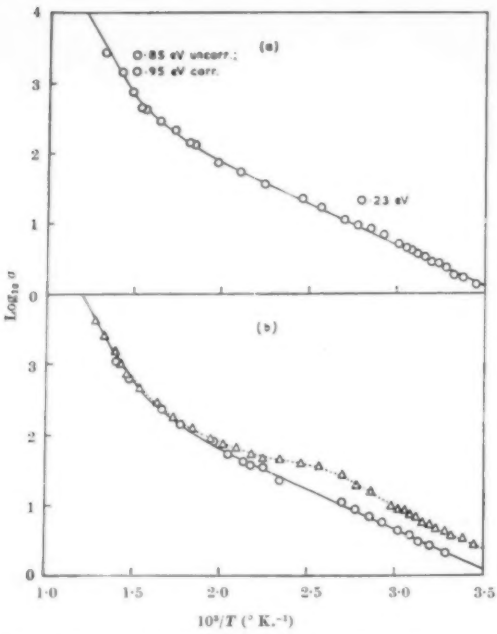

Fig. 1. Effect of ultra-violet irradiation on dark conductivity. *a*, Graph of log σ versus $1/T$ for an activated barium oxide coated cathode before ultra-violet irradiation; *b*, conductivity plots for the same cathode after ultra-violet irradiation: △, points plotted on warming cathode; ○, points plotted on cooling cathode. The conductance σ is expressed in arbitrary units

example given, the activation energy of the low-temperature process is 0·23 eV. and that of the high-temperature process is 0·95 eV., corrected for the parallel low-temperature component. On completion of the conductivity determination the cathode was irradiated through the quartz window with light from a high-pressure mercury arc, containing a high proportion of ultra-violet light, for 1 hr. The dark conductance of the cathode was then redetermined, starting at room temperature and heating the cathode. The effect of the ultra-violet irradiation was to increase the room-temperature dark conductance by a factor of about 2, and as the temperature was increased to about 370 °K. the enhancement increased slightly. From 370 °K. to about 550 °K. it decreased, and at temperatures above about 550 °K. the conductance was the same as that obtained at corresponding temperatures before irradiation. Heating was continued to about 800 °K., and the cathode was then cooled to room temperature. Conductance measurements taken during this process exactly reproduced the values obtained in the initial pre-irradiation determination. The results of this experiment are displayed in Fig. 1*b*; the curve obtained on warming the cathode immediately after irradiation is plotted as a broken line, and that taken on cooling as a continuous curve. For these plots separate current-voltage characteristics were not traced at each temperature, but the conductance was determined approximately, as indicated above. It was found that although the general form of this enhancement of dark conductivity is reproducible, the magnitude of the effect depends on a number of factors, such as the length of ultra-violet irradiation, the temperature of the cathode during irradiation, the period between the end of irradiation and the beginning of conductance measurements, and the rate of heating of the cathode. It was found that any enhancement could be completely removed by heating the cathode to about 600 °K. for a few minutes; on cooling, the conductance would return to the value obtained before irradiation.

This effect may be explained by postulating the existence of normally vacant donor-levels just below the conduction band in the energy-band model for barium oxide. Irradiation of the cathode sample with ultra-violet light may cause the excitation of electrons from the filled band and the occupation of some of these levels. Subsequent warming of the cathode in the dark in the course of conductivity determinations causes thermal excitation of electrons from these occupied levels to the conduction band, and an enhanced conductivity current is observed. Experiments on the enhancement of photo-electric emission by ultra-violet illumination[1–3] have led to the conclusion that such a band of normally unoccupied levels exists at about 2·5 eV. below vacuum potential. Although it is tempting to associate these levels with the enhancement of dark conductivity observed in the present work, there is, as yet, no direct evidence for the validity of such an association.

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A Clear Instance of Rheopectic Flow

We have found an instance of rheopectic flow induced by shear in a rotational viscometer. The material exhibiting this rare type of non-Newtonian flow behaviour was a viscous suspension prepared by mixing kaolin clay, precipitated calcium carbonate, oxidized starch, a styrene–butadiene latex, sodium pyrophosphate and water. The total solids of the suspension amounted to 65 per cent.

Characterization of the material as rheopectic was based on shearing stress against time data obtained under conditions of constant shear-rate. A set of these data is presented in Fig. 1. The stress is seen to increase with time until it becomes measurably independent of it. It was ascertained that laminar flow prevailed during measurements.

It appears that this shear-thickening effect is due largely to the presence of calcium carbonate since, when it was replaced by an equal mass of clay, the flow behaviour of the suspension was that of a typically thixotropic material. The longest dimension of the pigment particles in the suspension was smaller than 2μ for the clay and 0·3μ for the calcium carbonate. Clay and calcium carbonate particles were mostly anisometric (length to width ratio for clay about 1·5; for calcium carbonate about 4·0) while the latex particles were essentially spheres (average diameter 0·2μ).

Earlier observations of the rheopectic phenomenon have been mostly visual, qualitative, and confined to certain types of colloidal systems, usually sols[1–3].

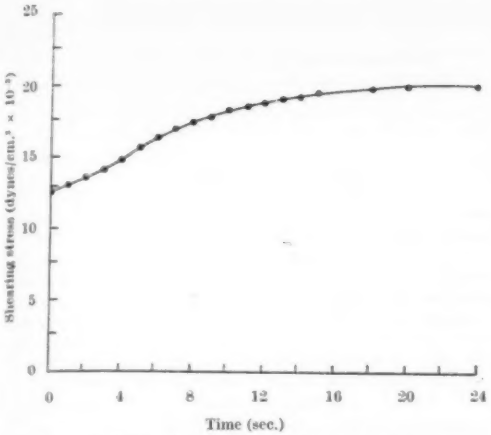

Fig. 1. Variation of shearing stress with time under conditions of constant shear-rate (1.850 sec.$^{-1}$) at 25 ± 0·2° C.

As a probable result of this, an assertion has been made recently that rheopexy is a flow phenomenon where structure build-up is brought about by small shearing-rates only[6], presumably less than about 10 sec.$^{-1}$. However, instances of rheopectic flow have been reported in the literature[7,8] although absolute values of shear-rate at which the phenomenon exhibits itself are not mentioned. Our observations demonstrate that rheopectic flow can develop at moderately high shear-rates (1,500–2,200 sec.$^{-1}$).

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METALLURGY

Scattering of Long Wave-length Neutrons by Irradiated Beryllium Oxide

Work on the scattering of long wave-length neutrons by irradiation-induced defects have been carried out by Antal, Weiss and Dienes for graphite[1] and by Antal and Goland for alumina[2]. The method depends on the fact that in materials of low neutron absorption cross-section, neutrons of wave-length greater than the Bragg cut-off are attenuated principally by inelastic scattering (for which the cross-section is a few hundred millibarns) and by the scattering from defects. Application of Babinet's principle shows that each isolated point defect introduces an amount of scattering equal to the free atom cross-section of the material.

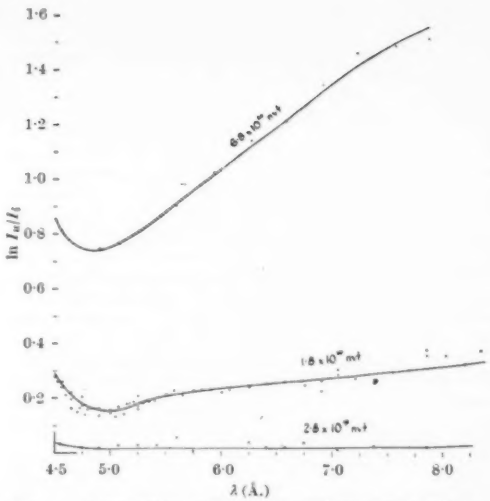

Fig. 1. Transmission ratios versus neutron wave-length for various neutron doses. Top curve, 6·8 × 10^{18} n.v.t.; middle curve, 1·8 × 10^{18} n.v.t.; ; bottom curve, 2·8 × 10^{19} n.v.t.

When the defects are randomly distributed a comparison of the attenuation by identical specimens before and after irradiation can give a direct measurement of the defect concentration. However, if the defects are clustered, interference occurs between the amplitudes of neutrons scattered from different defects within a cluster. In this case it is no longer possible to obtain an unambiguous estimate of the number of defects since the extra attenuation is dependent on wave-length, and the scattering is determined by the number of defects and the configuration of a cluster.

As part of an investigation of the behaviour of beryllium oxide under neutron irradiation, experiments have been carried out on specimens of this oxide irradiated to three different doses in *Hifar*.

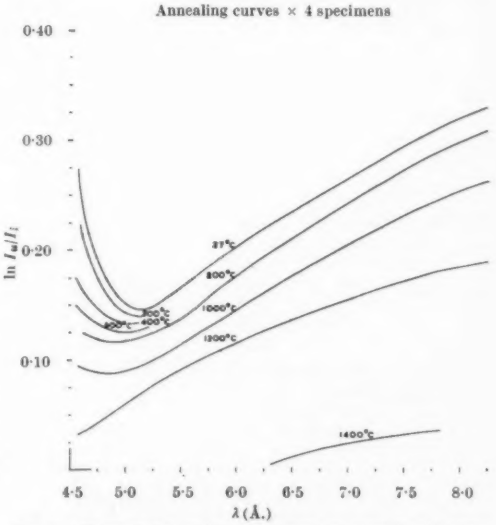

Fig. 2. Transmission ratios versus neutron wave-length after annealing at various temperatures

The neutron spectrometer used was similar to that described by Antal and Goland[2]. A mica monochromator was used with beryllium and beryllium oxide filters. The results are shown in Fig. 1 plotted as $\ln\left(\frac{I_u}{I_i}\right)$ versus wave-length, where I_u is the number of neutrons transmitted by 14 cm. of unirradiated beryllium oxide and I_i is the number transmitted by an identical specimen after irradiation. The doses quoted are the integrated doses over the fission neutron spectrum. It can be seen that the amount of extra scattering due to defects increases with neutron dose. For one set of specimens an annealing study was made by repeating the measurements after annealing the specimens at 200° C. intervals from 200–1,400° C. These results are shown in Fig. 2.

It can be seen that there are two distinct effects, a decrease in attenuation near the Bragg cut-off, which anneals steadily from 200° C. to 1,000° C., and an increase at longer wave-lengths, which anneals between 1,200° C. and 1,400° C.

The initial decrease in extra scattering near the cut-off wave-length could be explained if irradiation broadened the Bragg reflexions. However, this seems unlikely because the X-ray line broadening appears to be insufficient to account for the effect and this line broadening does not anneal until 1,400° C. A defect configuration which would explain the sharp decrease with wave-length is that proposed by Huntington[3] for copper, in which two interstitial atoms appear at each side of a vacant lattice site with an I–V spacing of less than 1 Å.

The theory developed by James[4] for the scattering of X-rays by polyatomic gases has been applied to the increase in scattering observed at long wave-lengths. A model which fits the observed dependence on wavelength is one in which the vacancies are clustered into close-packed groups containing 14 vacancies and the interstitials are randomly distributed. Values for the number of defects have been calculated on this model and these are shown in the Table 1.

Table 1

Specimen	Mean fission neutron dose (n.v.t.)	Irradiation temperature	Fraction of primary collisions	Fraction of displaced atoms	Displacements per primary collision
X22	$2{\cdot}8\times10^{19}$	70–75° C.	7×10^{-3}	$4{\cdot}5\pm4{\cdot}0\times10^{-4}$	1–13
X4	$1{\cdot}8\times10^{20}$	80–100° C.	$4{\cdot}5\times10^{-4}$	$4{\cdot}5\pm0{\cdot}4\times10^{-3}$	9–11
X39	$6{\cdot}8\times10^{20}$	80–100° C.	$1{\cdot}7\times10^{-3}$	$2{\cdot}2\pm0{\cdot}1\times10^{-3}$	12–14

The number of primary collisions was calculated by assuming a cross-section of 2·5 barns as the average of beryllium and oxygen atoms over the fission spectrum.

Further work on X-ray line broadening and other physical properties are being carried out in order to obtain more information on the nature of the defects.

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RADIATION CHEMISTRY

Radiation-induced Formation and Reactions of the Formyl Radical Ion

We have recently reported the formation of the CO_2^- radical ion and the related CO_2H radical by the action of ionizing radiation on aqueous solutions of carbon dioxide and bicarbonate[1].

It seemed of interest, therefore, to examine the corresponding reaction with carbon monoxide which should lead, in the first instance, to the CO^- radical ion and the COH radical. Some direct proof for the presence of CO^- in aqueous solutions has been obtained now from experiments in which oxygen-free aqueous solutions containing suitable organic solutes have been irradiated with ionizing radiations in the presence of carbon monoxide. It could be demonstrated that in certain cases the CO^- ion (or the COH radical respectively) could be combined with an organic radical with the formation of the corresponding formylated compound.

For example, the irradiation of aqueous solutions of methanol (10^{-1} *M*) in the presence of carbon monoxide (1 atm.) with Co-60 γ-rays ($1{\cdot}4\times10^{19}$ eV. ml.$^{-1}$) gave glycolaldehyde with a yield of $G = 0{\cdot}2$ (molecules/100 eV.) at pH = 5·4. The glycolaldehyde was identified by the formation of a coloured derivative with the 2 : 3-diaminophenazine reagent in concentrated sulphuric acid[2].

It is again important that the reaction is carried out in deoxygenated solutions because otherwise the negative polarons $(H_2O)^-$, which are the reducing entities[3] produced by the action of radiation on water, may be scavenged to a greater or lesser extent by the molecular oxygen according to:

$$(H_2O)^- + O_2 \rightarrow O_2^- + H_2O$$

In general, this formylation process depends on the formation of a free radical from the organic solute by the action of the radiation-produced OH or (H_2O^+), for example, in the case of methanol:

$$CH_3.OH + OH \rightarrow CH_2.OH + H_2O \qquad (1)$$

and association of the organic free radical with the COH (CO^-) according to the reaction:

$$CH_2.OH + COH \rightarrow CH_2.OH.CHO \qquad (2)$$

the formyl radical being formed by the reduction of CO according to:

$$CO + (H_2O)^- \rightarrow CO^- + H_2O \qquad (3)$$

$$CO^- + H^+ \rightleftarrows COH \qquad (4)$$

In this way it should be possible to produce some ^{14}C-carbonyl compounds labelled in the CO group.

We wish to thank the Rockefeller Foundation for financial support; one of us (J. H.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

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A Reversible Photochromic Polymer

ULTRA-VIOLET irradiation of chromium, molybdenum or tungsten hexacarbonyls dissolved in donor solvents causes the substitution of one or more molecules of carbon monoxide by solvent. The products are yellow in colour[1].

Methyl methacrylate polymers which contain occluded metal hexacarbonyls behave similarly. On ultra-violet irradiation or on being placed in sunlight, the polymers assume a deep yellow colour when as little as 0·1 per cent of, for example, chromium hexacarbonyl is present. At room temperature, either in air or high vacuum, the colour fades over a period of about 4 hr. The colour vanishes rapidly on warming to 100° but is stable when the specimen is stored in liquid nitrogen.

We believe that, on irradiation, the chromium hexacarbonyl decomposes to give $Cr(CO)_5$ and carbon monoxide. The former molecule may exist as such in the coloured polymer or may be stabilized by interaction with neighbouring ester groups or occluded carbon–carbon double bonds. Since the hexacarbonyl is trapped in the polymer matrix, the carbon monoxide cannot escape even on pumping, and at room temperature it slowly returns to the metal, producing the original colourless hexacarbonyl. Heating to 100° relaxes the polymer matrix and facilitates the reversion to hexacarbonyl.

The infra-red spectrum of the yellow polymethyl methacrylate showed a carbonyl shift of about 40 $cm.^{-1}$ to higher wave-lengths compared with unirradiated samples. No electron spin resonance spectrum other than that normally associated with polymethyl methacrylate irradiated with ultra-violet could be detected. These facts are consistent with the presence of $Cr(CO)_5$ or its addition products.

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CHEMISTRY

Fluoride-compensated Substitution of Bivalent Cations in the Magnetoplumbite Structure

$BaAl_{12}O_{19}$ has the structure of the mineral magnetoplumbite[1]. In the hexagonal unit cell, containing two formula units, the aluminium ions are distributed among five crystallographic positions. There are two tetrahedrally co-ordinated positions ($4f$ in C_{3v}/mmm) nine octahedrally co-ordinated ($2a$, $12k$, $4f$) and one ion surrounded by a trigonal bipyramid ($2b$ in C_{3v}/mmm) of oxide ions per formula unit.

The object of this work was to try to introduce bivalent cations, compensated by other foreign ions, in place of the Al^{3+} ions in the structure, in order to study the site preference of these ions in this structure. Cationic compensation (substitution of La^{3+} for Ba^{2+}) and anionic compensation (substitution of F^- for $O^=$) were both used. The latter method was first used for this structure by Frei *et al.*[2], who introduced fluoride into $BaFe_{12}O_{19}$ with concurrent reduction of some Fe^{3+} to Fe^{2+}.

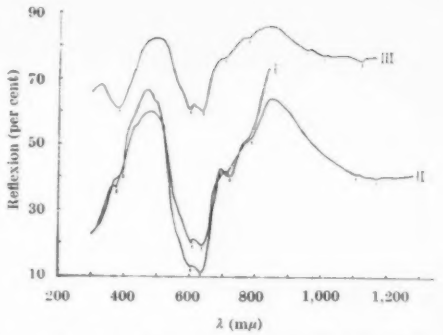

Fig. 1. Per cent reflexion versus wave-length, mμ (magnesium carbonate standard). I, $BaNi_{1-x}Al_{19-x}O_{17-x}F_{2-x}$; II, $BaNi_{2-x}Al_{9-x}O_{16-x}F_{2-x}$; III, $BaZn_2Ni_{9-x}Al_{9-x}O_{16-x}F_{2-x}$

Using cationic compensation, materials having the general formula $Ba_{1-x}La_xNi_xAl_{12-x}O_{19}$ were prepared. For samples where $x \leqslant 0{\cdot}2$, X-ray diffraction indicated the presence of only the magnetoplumbite phase. At values of $x \geqslant 0{\cdot}3$ a mixture of $LaAlO_3$, Al_2O_3 and magnetoplumbite was found. The samples where $x \leqslant 0{\cdot}2$ were blue in colour; reflectance spectra, taken on pellets pressed from the powders, give the following absorption peaks:

$cm.^{-1}$	Intensity	Site
27,000	Very weak	Octahedral
17,700	Strong	Tetrahedral
16,400	Very strong	Tetrahedral
15,850	Very strong	Tetrahedral
13,950	Medium	Tetrahedral
12,900	Very weak	Tetrahedral

All these absorptions, except the first one, are in good agreement with the spectrum of Ni^{2+} in ZnO (ref. 3) and indicate that almost all the Ni^{2+} is occupying tetrahedral sites. The presence of a small amount of Ni^{2+} in octahedral sites is indicated by a weak absorption at 27,000 $cm.^{-1}$, which is in good agreement with the strongest absorption in $Ni(H_2O)_6^{2+}$ (ref. 3).

Due to the breakdown of the magnetoplumbite phase at $x \geqslant 0{\cdot}3$, the second method of charge compensation was attempted in order to examine the site preferences as a function of concentration of the bivalent ion.

Materials having the general formula $BaM^{II}Al_{12-x}O_{19-x}F_x$ were prepared by firing mixtures of BaF_2, $BaCO_3$, MO, Al_2O_3 at 1,350–1,400° C. in an atmosphere of dry nitrogen gas.

The first system to be investigated was one in which $M^{II} = Ni^{2+}$ and $x = 0{\cdot}1$, $0{\cdot}2 \rightarrow 1$, $1{\cdot}5$, $2{\cdot}0$, $2{\cdot}5$. $BaNiZnAl_{10}O_{18}F_2$ and $BaNi_{0.5}Zn_2Al_{9.5}O_{16.5}F_{2.5}$ were also prepared, using AlF_3 where an extra source of fluoride was needed. X-ray diffraction showed that for all samples where $x \leqslant 2{\cdot}5$, only the hexagonal phase was present; where $M^{II} = Ni^{2+}$ and $x \geqslant 1{\cdot}0$ the c-axis was doubled. This effect did not appear where $M^{II} = Zn^{2+}$ or Co^{2+}. At values of $x > 2{\cdot}5$, the hexagonal phase disappears and is replaced by a spinel phase, probably $Ni_{1+x}Al_{2-x}O_{4-x}F_x$, in the residue after leaching with hot concentrated hydrochloric acid.

The reflectance spectra of the materials the general formula of which is $BaNi_xAl_{19-x}O_{19-x}F_x$, where $x \leqslant 2{\cdot}0$, are identical with the spectra described previously. This indicates that, when substituted for up to 2 gm.-atoms of Al^{3+} (in $BaAl_{12}O_{19}$), Ni^{2+} strongly prefers tetrahedral sites (Fig. 1, curve I).

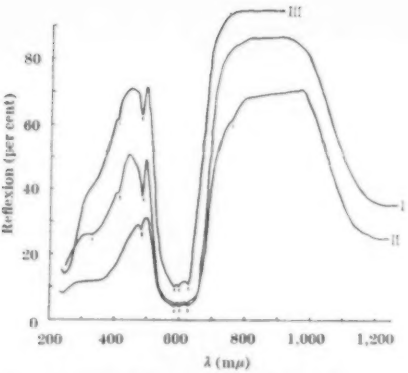

Fig. 2. Per cent reflexion versus wave-length, mμ (magnesium carbonate standard). I, $BaCo_2Al_{10}O_{17}F_2$; II, $BaCo_{0.5}Al_{9.5}O_{16.5}F_{2.5}$; III, $BaZn_2Co_{0.5}Al_{9.5}O_{16.5}F_{2.5}$

At $x = 2{\cdot}5$ (Fig. 1, curve II) a marked increase is observed in the absorption at 27,000 cm.$^{-1}$, indicating an increase in the amount of Ni^{3+} in octahedral sites. A weak absorption is found at 8,900 cm.$^{-1}$ which also indicates octahedral nickel. The increase in octahedral nickel is caused by the excess Ni^{2+} (greater than 2 gm.-atoms) which is forced to occupy the octahedral or trigonal bipyramid sites. The tetrahedral sites are saturated by the first 2 gm.-atoms of Ni^{2+}. All these samples were blue in colour. Fluoride analysis of $BaNiAl_{11}O_{18}F$ yielded a value of 2·2 per cent by weight of fluoride. The calculated value is 2·0 per cent.

The sample $BaZn_2Ni_{0.5}Al_{9.5}O_{16.5}F_{2.5}$ was prepared in order to see if Zn^{2+} (which has a strong tetrahedral site preference) could displace Ni^{2+} from the tetrahedral sites. Fig. 1, curve III, shows a large increase in the amount of octahedral Ni^{3+} with a corresponding decrease in the amount of tetrahedral Ni^{2+}. This sample had a light blue-green colour.

The next system to be investigated was one in which $M^{II} = Co^{2+}$ and $x = 0{\cdot}1$, 0·4, 1·0, 2·0, 2·5. $BaZnCoAl_{10}O_{17}F_2$ and $BaZn_2Co_{0.5}Al_{9.5}O_{16.5}$ were also prepared. X-ray diffraction showed the presence of only one phase (magnetoplumbite). The intensities of the absorptions, of course, vary with composition.

The spectra of all these samples were identical except for that of $BaCo_{2.5}Al_{9.5}O_{16.5}F_{2.5}$. This sample showed a strong absorption in the near ultra-violet region which may be caused by cobalt in octahedral sites. The observed absorption maxima for these materials are:

cm.$^{-1}$	Intensity	Site
24,400	Medium	Tetrahedral
20,900	Medium	Tetrahedral
17,100	Very strong	Tetrahedral
16,800	Very strong	Tetrahedral
16,100	Very strong	Tetrahedral

These values are in fair agreement with the spectrum observed for Co^{2+} in ZnO (ref. 4) and they are in good agreement with the values obtained from the crystal field splittings as a function of Dq which were calculated by Low[5]. Curve III (Fig. 2) shows the spectrum of $BaZn_2Co_{0.5}Al_{9.5}O_{16.5}F_{2.5}$. The absorption spectra indicate that Zn^{2+} does not displace Co^{2+} from tetrahedral sites to any appreciable extent.

It has been shown that Ni^{2+} and Co^{2+}, compensated by F^-, have tetrahedral site preferences, in $BaAl_{12}O_{19}$, in all concentrations up to the saturation of the tetrahedral sites. The preference of Co^{2+} for tetrahedral sites has been shown to be greater than that of Ni^{2+}, as tetrahedral Ni^{3+} is displaced by Zn^{2+} and tetrahedral Co^{2+} is not. The tetrahedral site preference of Ni^{2+} (which would normally have an octahedral site preference) may be caused by a Jahn–Teller stabilization. This Jahn–Teller effect may also be the cause of the change of symmetry in the hexagonal unit cell which effected a doubling of the c-axis. This effect was not observed with Co^{2+}.

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Ultrasonic Velocity in Aqueous Silver Nitrate Solution

We have confirmed the recent report[1] that, in the case of aqueous solutions of silver nitrate, the ultrasonic velocity decreases with increasing concentration over the range of concentration investigated. However, a minimum velocity is observed at approximately 4 M; thereafter, increasing concentration up to saturation causes a monotonic increase in ultrasonic velocity, as is usual for aqueous electrolyte solutions. Part of the complete velocity/molarity curve is shown in Fig. 1; these results were obtained with a variable-path fixed frequency (263·3 kc./sec.) interferometer, at 25° C.

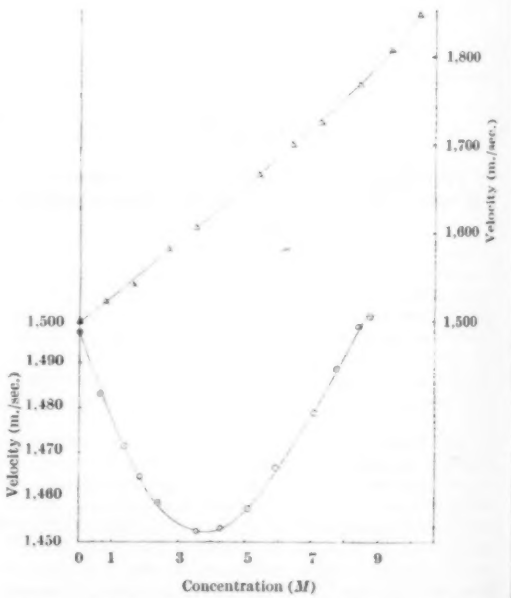

Fig. 1. Variation of ultrasonic velocity with concentration in aqueous solutions of electrolytes. ○, Silver nitrate; △, ammonium nitrate

Although the suggested explanations[2-4] in terms of heavy radicals or complex ions may be correct, it is clear that the parameters directly responsible for the minimum velocity are the density, and the adiabatic compressibility, β, of the solution. These are related by the equation:

$$v^2 = 1/\rho\beta$$

where v is the ultrasonic velocity. The variation in v with molarity may then be expressed:

$$dv/dc = -v/2.\ (1/\rho\ .d\rho/dc + 1/\beta.d\beta/dc) \quad (1)$$

The terms $d\rho/dc$ and $d\beta/dc$ are of opposite sign; hence the relative magnitudes of the terms within brackets on the right-hand side of equation (1) may produce monotonic increase or decrease of v with concentration, or, as in the case of silver nitrate, a minimum velocity.

It is interesting that ammonium nitrate solutions, which show similar variations with molarity of electrical conductance and of mean-ion activity coefficient to those of silver nitrate, do not exhibit a minimum ultrasonic velocity; the curve for ammonium nitrate is included in Fig. 1. Presumably the ion-pairing in these solutions[5] does not always result in a velocity minimum.

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Complexes of Vermiculite with Amino-Acids

Organic substances, either as neutral molecules or as cations, form 'sandwich' complexes with the expanding-lattice silicate minerals in which films of the organic substance a few Angstroms thick alternate with silicate layers 10 Å. thick. Recent work has shown that macroscopic vermiculite crystals complexed with butylammonium ions absorb large quantities of water and swell in the direction perpendicular to the plane of the silicate layers[1,2].

We have observed that macroscopic vermiculite crystals complexed with various amino-acids also swell anisotropically to many times their original size. Two apparently different swelling mechanisms are involved, depending on whether the amino-acid is present as the cation or as the dipolar ion; in both instances, swelling is confined to the direction perpendicular to the plane of the silicate layers (Fig. 1). In the jelly-like swollen crystals, the silicate layers remain effectively parallel to one another and, under optimum swelling conditions, may be some hundreds of Angstroms apart.

One type of swelling resembles that exhibited by the butylammonium–vermiculite complexes, and occurs after the amino-acid has been introduced as the interlayer cation replacing the magnesium or other inorganic cations present in the natural minerals. Treatment with 0·5 *M* aqueous solutions of the hydrochloride of the acid for two or three days at 80° C.

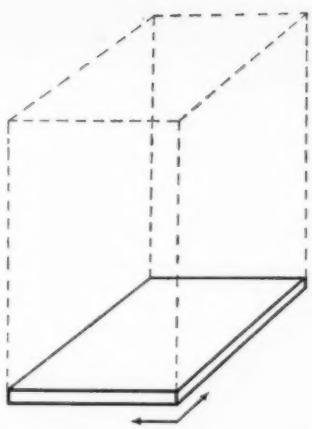

Fig. 1. Representation of the dimensional change in a vermiculite–amino-acid crystal before swelling (full line) and after swelling (dashed line). Silicate layer plane indicated by arrows

is usually sufficient for complete exchange with crack-free crystals of dimensions 1 mm. × 1 mm. × 0·1 mm. The amount of amino-acid taken up is close to the cation exchange capacity of the minerals, that is, in the range 120–180 m.equiv./100 gm. Swelling develops when the amino-acid solution, in which the crystals are immersed, is diluted to a critical value which varies with the amino-acid and the vermiculite used. Swelling increases with decreasing solution concentration and is maximal at infinite dilution. The initiation of swelling is thought to coincide with 'hydration' of the interlayer amino-acid cations[2] and their dissociation from the negatively charged silicate surfaces, while subsequent swelling appears to be osmotically controlled. This type of swelling has been observed with ornithine, lysine and γ-aminobutyric acid.

A second type of swelling occurs when natural vermiculite crystals are immersed in strong amino-acid solutions containing the acid in the dipolar form. In this type, replacement of the interlayer inorganic cations is inappreciable and swelling may take place at room temperature in a few minutes. Swelling develops only in solutions of sufficiently high concentration and increases directly with concentration. Considerable amounts of amino-acid are taken up into the interlayer regions of the mineral, the intracrystalline concentration being of the same order as that of the external solution. Here the dielectric constant of the immersion solution appears to be the controlling factor. The mechanism is thought to involve the masking of the charge on the interlayer cations so that the effective electrostatic attraction between them and the silicate layers is reduced. Swelling of this type has been observed with solutions of glycine, β-alanine, γ-aminobutyric acid and ε-aminocaproic acid.

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Semiconductivity of Chlorpromazine

It has been shown in ref. 1 that chlorpromazine, the common name for phenothiazine-2-chloro-10-(3 dimethyl aminopropyl), is "a quite extraordinarily strong electron donor"; it is "the first substance ever found in which, in its ordinary stable state, the highest filled level corresponds to an antibonding orbital". It thus should be expected that chlorpromazine, also in view of its striking physiological activity, should exhibit some interesting electrical properties.

The parent compound, phenazin, has recently been shown[2] to be an intrinsic semiconductor with an activation energy of 2·1 eV., relating the conductivity σ to temperature T by:

$$\sigma = \sigma_0 \exp - E/2kT$$

We have examined the resistance of the purified substance, melting point 56·85° C., kindly supplied to us by Messrs. May and Baker, Ltd., Dagenham, Essex, England, in the form of glass-like slabs cast from the melt in a 'Perspex' former between roughened platinum electrodes. A thermistor bead was also cast into the slab, but located outside the field, to act as a thermometer. The whole apparatus was placed under thermostatical control. The electrical resistance was found to drift downwards for 20–30 min. after the application of an electric field. Once equilibrium was attained, very little further drift was observed during a period of 3 hr. A time-interval of 20–30 min. was therefore allowed between each reading. The voltage applied was 85 V. d.c.

Data of resistance against temperature were obtained on fifteen runs of heating and cooling cycles, referring to four different slabs. The temperature coefficient of the resistance R was found to reverse sign at 32° C., becoming negative at higher temperatures. A plot of log R against $1/T$ of this transition region is shown in Fig. 1, and a plot of the high temperature region between 34° and 80° C. is shown in Fig. 2.

Chlorpromazine thus appears to be an impurity semiconductor at low temperatures, and an intrinsic semiconductor at temperatures in excess of about 32° C. There is no significant change in either resistivity or value of E on fusion; E remains substantially constant at 2·1 eV. at temperatures between

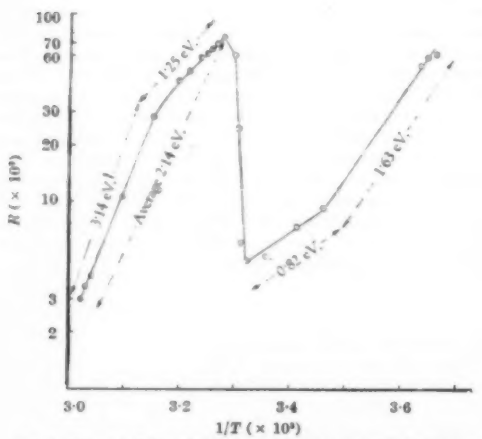

Fig. 1. The resistivity of chlorpromazine, plotted on a logarithmic scale against inverse temperature, showing the transition at 32° C.

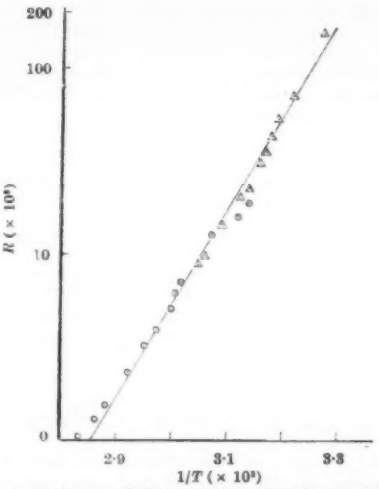

Fig. 2. Resistance of chlorpromazine above the transition. This plot covers the region between 34° and 80° C., the melting point being 56·85° C. There is no significant discontinuity on fusion, nor hysteresis between heating and cooling cycles. △, Heating cycle; ○, cooling cycle

34° and 80° C. The region of positive temperature coefficient connecting the impurity conductance with the intrinsic conductance regions is considered to be a régime where the concentrations of localized donor- or acceptor-levels nearly equal the concentration of charge carriers. The transition at 32° C. is believed to be associated with a crystallographic change, the substance existing in different modifications below and above that temperature.

There is no evidence for a hysteresis. The points referring to both the heating and the cooling cycle, shown in Fig. 2, are seen to fall on one single straight line.

The conductivity with direct current of chlorpromazine at the transition temperature of 32° C. was found to be $9{\cdot}2 \times 10^{-11}$ (ohm-cm.)$^{-1}$. The value of E found for chlorpromazine, namely, 2·1 eV., agrees with that reported[2] for phenazine; this conforms with ideas put forward[3] on the role of the delocalization of the π-electrons in a ring system. The phthalocyanines are also rather insensitive, so far as E is concerned, to modifications of the molecule which leave the ring structure substantially unaltered, as, for example, by the chelation of metal atoms[4].

Curves closely resembling that shown in Fig. 1 have been reported in ref. 4 for glassy, amorphous selenium. This element also exhibits no change in E on fusion.

Measurements of permittivity and of loss factor at audio- as well as at radio-frequency confirmed the existence of the transition at 32° C.

Direct proof of the existence of a crystallographical change in that region of temperature has been obtained by taking infra-red spectra at temperatures of about 25° C., 36° C. and 55° C.; these temperatures are approximate only, due to heating of the sample by radiation. While no change appeared between the spectra obtained at the two higher temperatures, pronounced differences were observed between those obtained at the two lower temperatures. The changes affect the side-chain as well as the ring system, and are consistent with a steric change in the alignment and the co-ordination of the molecules.

We wish to thank Messrs. May and Baker, Ltd., Dagenham, Essex, for the supply of chlorpromazine, and Dr. R. L. Werner of this Department for his assistance in the interpretation of the infra-red spectra.

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Structure of δ-Plutonium

In a recent article[1] a modification to the normally accepted close-packed face-centred cubic structure of δ-plutonium was suggested. The purpose of this was to give a continuous gradation, with increasing temperature, in the amount of covalency in the various plutonium structures; from this was deduced a predicted value of the negative expansion coefficient of about the correct size.

The proposed modification consisted of slight displacements inwards of the face-centring atoms in alternate cells. This structure should give additional weak 'superlattice' lines on an X-ray diffraction pattern in addition to those normally observed. However, we could not detect these experimentally, although calculations showed that the X-ray diffractometer techniques used were amply sensitive. The absence of these lines does not lend credence to the proposed structure.

A full description of the experiments is to be published elsewhere.

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Girard *T* Reagent for Carbonyls

The water-soluble properties of Girard *T* hydrazones make the reagent potentially effective for isolation of carbonyls from mixtures soluble in non-polar solvents. In work on the chemistry of rancidity[1], we have found the reagent useful in learning about the distribution of volatile and non-volatile carbonyls in rancid fat. This work indicated possible usefulness of the reagent for investigation of the complex carbonyl anatomy of autoxidized fats. Successful application depends on adaptation to quantitative isolation of very small amounts of carbonyls, and on devising means of controlling the reaction so as to isolate at will free carbonyl and bound or potential carbonyl.

This report deals with certain aspects of the methodology of the Girard reaction. In experiments with known amounts of monocarbonyls, 0·5–1·0 gm. of Girard *T* reagent was reacted in 20·0 ml. of purified anhydrous methanol or ethanol and shaken 3 hr. at room temperature, or refluxed for 1 hr. The reaction mixture was poured into 200 ml. of ice water, and then extracted with petroleum ether to remove non-carbonylic material. The aqueous phase contained the Girard *T* hydrazones. These were converted directly into 2,4-dinitrophenylhydrazones by treatment with 30 ml. of a saturated 2,4-dinitrophenylhydrazine solution in 2 *N* hydrochloric acid[2] and 190 ml. of water. After standing overnight at room temperature, or heating 1 hr. on the steam bath, the 2,4-dinitrophenylhydrazones were removed by successive extractions with carbon tetrachloride and benzene. Monocarbonyl hydrazones were separated by passage of extract residues through hydrated alumina[2]. Total amounts of these compounds were determined by measurement of absorbance at the wave-length of maximum absorption[2–6] in 100 ml. of carbon tetrachloride solution. In the case of mixtures, paper chromatographic methods are available for resolution, identification, and estimation of monocarbonyls[3–6].

Table 1. Blank Determinations

Girard *T* reagent	2,4-Dinitrophenylhydrazine		Ethanol	Tertiary butyl alcohol	No solvent
—	RT*	1 hr.	0·048	—	—
—	RT	24 hr.	0·130	0·024	—
—	Heated	1 hr.	0·480	—	—
0·5 gm. (unpurified) RT, 3 hr.	RT	24 hr.	—	0·250	—
1·0 gm. (unpurified) RT, 3 hr.	RT	24 hr.	0·850	0·518	—
0·5 gm. (purified) RT, 3 hr.	RT	24 hr.	0·338	0·050	—
0·5 gm. (purified) Reflux, 1 hr.	RT	24 hr.	0·478	0·120	—
0·5 gm. (purified) Reflux, 2 hr.	RT	24 hr.	0·900	—	—
0·5 gm. (purified) RT, 3 hr.	Heated	1 hr.	2·63	0·813	0·087
0·5 gm. (purified) RT, 3 hr.	Heated	1 hr.	—	0·310†	—

* Room temperature.
† Purified by treatment with 2,4-dinitrophenylhydrazine and hydrochloric acid.

The above procedure for the Girard reaction gave high blanks which seriously affected determinations of very small amounts of monocarbonyls. The Girard *T* reagent was found to contain hydrazone, which could be removed by several recrystallizations under mild conditions from ethanol. However, the greater part of the blank was found to result from a curious reaction between the primary alcohols used as solvents and both the Girard *T* and 2,4-dinitrophenylhydrazine. The amount of formaldehyde or acetaldehyde hydrazone formed was related directly to the severity of reaction conditions and independent of exposure to air. Apparently, this carbonyl formation is a dehydrogenation and analogous to the formation of osazones from α-keto alcohols. The reaction has been studied on a very large scale by Braude and Forbes[7], who determined that it resulted in a 5–25 per cent yield with primary and secondary alcohols containing two or more ethylenic or phenyl substituents in conjugation with the alcohol group. These investigators stated that derivatives were not formed with monophenyl, α,β unsaturated and saturated alcohols. This has been found not to be completely correct in the case of the two saturated primary alcohols used.

Methanol and ethanol have been the standard solvents used in the Girard *T* reaction since it was first reported[8]. Tertiary butyl alcohol is proposed as a relatively inert solvent for use in the presence of the Girard *T* reagent and 2,4-dinitrophenylhydrazine. The Girard *T* reagent has limited, but adequate, solubility in this alcohol, which is a much better solvent for fats than methanol or ethanol. Blanks found with ethanol and tertiary butyl alcohol are

shown in Table 1. Ethanol blanks were substantial, and increased with vigour of conditions of reaction. Both Girard *T* and 2,4-dinitrophenylhydrazine are shown to act on ethanol. Purified butyl alcohol gave negligible blanks when the Girard *T* reagent was recrystallized, except when heated in the 2,4-dinitrophenylhydrazine step. Purification of tertiary butyl alcohol with 2,4-dinitrophenylhydrazine and hydrochloric acid reduced this blank somewhat. This may indicate the presence of a persistent impurity, possibly a ketal. In any event, heating in the 2,4-dinitrophenylhydrazine step was unnecessary for complete conversion.

The use of tertiary butyl alcohol in the Girard *T* reaction gave nearly quantitative results, under mildest conditions of reaction, with *n*-alkanals, *n*-alk-2-enals, and *n*-alk-2,4-dienals. Reaction of methyl ketones with the Girard *T* reagent was less complete (about 80 per cent).

The entire suitability of the Girard *T* reagent for study of total carbonyls in autoxidized fat remains to be determined. Little is known about its reaction with dicarbonyl compounds. In view of its dehydrogenation action on primary and secondary alcohols, the reagent might convert hydroxy compounds produced in the autoxidation into carbonyls. Direct reaction of Girard *T* reagent or 2,4-dinitrophenylhydrazine with fat autoxidation products could result in higher total carbonyl than actually present. There has been some indication that this may occur; such as continual removal of carbonyl on repeated treatments with Girard *T* reagent[1], but further study is necessary. It was considered that the information in this report might be of use to those interested in applications of the Girard *T* reagent.

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BIOCHEMISTRY

Immunochemical Distinction between Insulins with Identical Amino-Acid Sequences

Virtually all human subjects treated with commercial mixtures of beef-pork insulin develop circulating insulin-binding antibodies that are readily detectable with insulin labelled with iodine-131 by means of paper or starch electrophoresis, paper chromatography or ultracentrifugation[1]. These antibodies react not only with the specific immunizing insulins but also with horse, sheep and human insulins[2], and with dog, monkey, rabbit and whale insulins (unpublished work) in greater or lesser degree. Harris, Sanger and Naughton[3] have shown that the amino-acid sequences of insulins from four different ungulate species (cattle, pig, horse and sheep) differ only in the 8–10 positions of the *A* chains. Although some human antisera react quite similarly

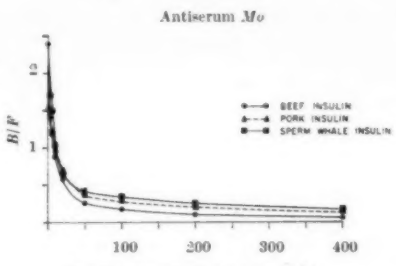

Fig. 1. Ratio of antibody-bound beef insulin-131 to free beef insulin-131 (*B*/*F*) as a function of the concentration of unlabelled crystalline insulins from various animal species. Human antiserum *Mo* at 1 : 10 dilution

with these four insulins, certain antisera can distinguish clearly among insulins from the different species, generally reacting much more strongly with beef and sheep insulins than with pork and horse insulins[2]. These differences in reaction of the ungulate insulins have been related[2] to the known differences in the 8–10 sequence of the *A*-chain or to possible differences in secondary or tertiary structure.

Harris *et al.*[3] found the amino-acid sequences of a whale insulin sample to be identical with those of pork insulin. More recently, Ishihara, Saito, Ito and Fujino[4] established that these sequences are present in sperm whale insulin but differ slightly from those of sei-whale insulin.

The present communication describes experiments showing that certain insulin antisera can discriminate sharply between the competitive inhibition offered by pork and sperm whale insulins to the binding of beef insulin labelled with iodine-131 to combining sites on the antibody molecules.

Crystalline beef insulin labelled with iodine-131 was prepared by methods described previously[1]. Mixtures containing identical trace concentrations of beef insulin-^{131}I, different concentrations of unlabelled crystalline beef, pork or whale insulin and identical concentrations of antiserum (added last in all cases) were prepared at room temperature and allowed to stand for 4 days at 4° C., at the end of which time they were analysed by paper chromato-electrophoresis[1] for the percentage of insulin-^{131}I bound to antibody. Briefly, this determination depends on the adsorption of unbound ('free') insulin-^{131}I to the paper at the site of application[1,5] and the migration of antibody-bound insulin-^{131}I with the inter β-γ globulins[1,6]. The ratio of bound to free insulin-^{131}I is plotted as a function of the concentration of unlabelled insulin present in the mixtures.

Many antisera react quite similarly with crystalline beef, pork and sperm whale insulins. Antiserum from subject *Mo* (Fig. 1) is typical of this group of cases in which not more than about 1·5 times as much sperm insulin as pork insulin was required to reduce the ratio of bound to free insulin-^{131}I for beef insulin-^{131}I to any specified level. Similar results have been observed with antiserum from a guinea pig immunized with beef insulin. In contrast to this relative indifference to the species from which the insulin comes are the results obtained with antisera *Le* (Fig. 2, upper) and *Ge* (Fig. 2, lower), which discriminate sperm whale insulin sharply from pork insulin. More than 150 times as much sperm whale insulin as pork insulin was required to reduce the ratios of bound to free insulin-^{131}I (beef insulin) to

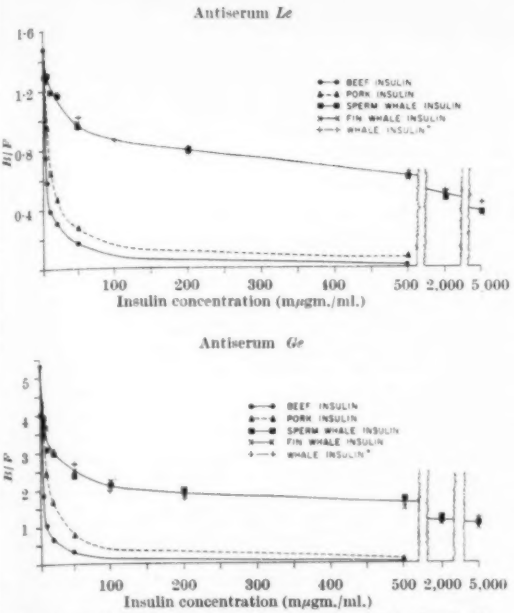

Fig. 2. Ratio of antibody-bound beef insulin-131 to free beef insulin-131 (B/F) as a function of the concentration of unlabelled crystalline insulins from various animal species. Upper: 1 : 60 dilution of antiserum *Le*; lower: 1 : 400 dilution of antiserum *Ge*. Whale insulin—unidentified as to species, see text

certain levels in *Le* and more than 100 times as much in *Ge*.

Experiments illustrated in Figs. 1 and 2 were carried out simultaneously with the same preparations and the results with antisera *Le* and *Ge* were confirmed repeatedly in several experiments with different preparations of insulin-^{131}I and different dilutions of antiserum and concentrations of insulin.

The differences observed between sperm whale and pork insulins in antisera *Le* and *Ge* can clearly not be attributed to any inherent inactivity or other anomaly of the sperm whale insulin crystals, in view of the essential similarity of reactions of the two insulins in many antisera as typified by antiserum *Mo* (Fig. 1). Furthermore, the procedure employed in purification of the insulin appears to be of no consequence, since three different preparations of beef insulin have been shown to react identically with an antiserum that clearly recognized species differences[2].

Fig. 2 also shows results with fin whale insulin and with a 'whale' insulin kindly supplied by Dr. A. M. Fisher, Connaught Laboratories, Toronto. The latter is not unequivocally identified as to species but may have been the same preparation analysed by Sanger and colleagues. The similarity of reactions of sperm whale and fin whale insulins suggests that these two insulins may have the same structure, but does not establish their identity with respect to amino-acid sequence since the immunochemical reaction does not always differentiate clearly between insulins with different sequences (cf. ref. 2 and Fig. 1). However, the marked discrimination between sperm whale and pork insulins by certain antisera is of considerable interest and would appear to provide the first evidence that two different proteins with identical amino-acid sequences (assuming the absence of any D-amino-acid configurations) may yet possess differences in secondary or tertiary structure. However, the observed species specificities entail differences in the standard free-energy change of binding of the order of only 3–4 k.cal. per mole, whereas the standard free-energy changes themselves are in the range of 8–12 k.cal. per mole[7]. Thus, a relatively small distinction in the structure of whale insulin at a critical site might well account for the lesser degree of complementarity to certain antibodies revealed by these investigations. If confirmed by more direct physico-chemical work, these findings can only mean that the overall protein structure is not determined by amino-acid sequence alone.

Finally, the possible significance of these observations to concepts of genetic transmission of inherited characteristics of protein synthesis is not to be overlooked. It would seem that, in addition to amino-acid sequence, the precise configuration of folding of the protein molecule is determined by the genetic apparatus.

We are indebted to Dr. O. Behrens, Lilly Research Laboratories, for supplies of crystalline beef and pork insulins and to Dr. T. Shibata, Taiyo Fishery Co., Yokosuka, Japan, through the kindness of Dr. Y. Ishihara, Hokkaido University, Japan, for supplies of crystalline sperm whale and fin whale insulins.

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Biosynthesis of Hydrastine

Little experimental work has been done on the biosynthesis of the phthalideisoquinoline, the benzylisoquinoline, and the protoberberine alkaloids. All these should be derived, according to biogenetic hypothesis, from two units of tyrosine[1] or two units of shikimic acid[2].

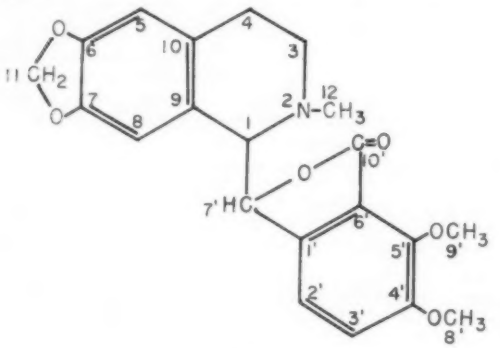
In extracts of *Berberis vulgaris* L. radioactivity from [2-^{14}C]-phenylalanine has been qualitatively detected by autoradiography in a chromatographic spot attributed to berberine[3]. In *Coptis japonica* Makino, DL-[2-^{14}C]-tyrosine gave rise to berberine containing radioactivity of unknown distribution in the nucleus of the alkaloid[4]. Activity from L-[U-^{14}C]-tyrosine was incorporated into papaverine, narcotine and narcotoline in *Papaver somniferum* L.[5]. The distribution of carbon-14 suggested that narcotoline was derived from two tyrosine units, but the evidence was indicative rather than conclusive. In the only detailed investigation to date Battersby and Harper[6] showed by unambiguous degradation of labelled papaverine obtained from DL-[2-^{14}C]-tyrosine in *Papaver somniferum* L. that two units of precursor were incorporated into the alkaloid without randomization.

We have examined the biosynthesis of hydrastine (I) in *Hydrastis canadensis* L. Mature plants were grown hydroponically in aerated nutrient solution[7]

Table 1

	Precursor		Duration of feeding (days)	Products			
				Hydrastine		Berberine	
	Total activity (counts/min.)	Specific activity (counts/min./mM)		Weight (gm.)	Specific activity (counts/min./mM)	Weight (gm.)	Specific activity (counts/min./mM)
DL-[2-^{14}C]-tyrosine	$8{\cdot}68 \times 10^7$	$5{\cdot}15 \times 10^5$	17	0·154	$5{\cdot}17 \times 10^4$	0·330	$12{\cdot}4 \times 10^4$
DL-[2-^{14}C]-tyrosine	$11{\cdot}0 \times 10^7$	$6{\cdot}55 \times 10^5$	8	0·128	$4{\cdot}47 \times 10^4$	0·172	$6{\cdot}52 \times 10^4$
DL-[2-^{14}C]-phenylalanine	$8{\cdot}87 \times 10^7$	$4{\cdot}18 \times 10^5$	8	0·405	$0{\cdot}06 \times 10^4$	0·472	$0{\cdot}42 \times 10^4$
D-[U-^{14}C]-glucose	$8{\cdot}75 \times 10^7$	$8{\cdot}75 \times 10^5$	17	0·506	$0{\cdot}41 \times 10^4$	0·612	$1{\cdot}27 \times 10^4$

containing DL-[2-^{14}C]-tyrosine. Hydrastine and berberine were extracted from the roots. Chemical and radioactive yields are recorded in Table 1. In parallel experiments DL-[2-^{14}C]-phenylalanine and D-[U-^{14}C]-glucose were administered. Radioactive incorporation of these into the alkaloids, also summarized in Table 1, was much less efficient. These results indicate that conversion of phenylalanine to tyrosine is minimal in *Hydrastis* (cf. ref. 8), and suggest that Wenkert's biogenetic hypothesis[3] is not valid in this case.



(I)

Hydrastine from plants grown with tyrosine-^{14}C for 17 days was degraded to locate the radioactivity, which was found, in accord with classical[1], but contrary to Wenkert's[3], biogenetic prediction, exclusively at carbon atoms 1 and 3. Oxidation gave inactive opianic acid (atoms 1′–10′) and active hydrastinine (atoms 1–12). The latter underwent Hofmann degradation to yield hydrastal (atoms 1, 3–11), which was oxidized to 6-vinylpiperonylic acid (atoms 1, 3–11). No loss of radioactivity occurred in these steps. Bromodecarboxylation, accompanied by modification of the vinyl group, gave radioactive 6-bromo-α-hydroxyhomopiperonylbromide (atoms 3–11) and radioactive carbon dioxide (atom 1). The latter was collected as barium carbonate, and then regenerated and converted to benzoic acid by treatment with phenylmagnesium bromide. The homopiperonylbromide derivative was oxidized to 6-bromopiperonylic acid (atoms 4–11), which contained little radioactivity. Radiocarbon was thus confined to carbons 1 and 3 of the hydrastine skeleton. The activities found in hydrastine and its degradation products are summarized in Table 2. The degradation sequence clearly demonstrates that tyrosine serves as a specific precursor of hydrastine, and that, as predicted, two tyrosine units participate in the biosynthesis of the alkaloid.

Table 2

Compound	Atoms of hydrastine	Specific activity (counts/min./mM)	Relative activity
Hydrastine		$5{\cdot}17 \times 10^4$	100
Hydrastinine	1–12	$5{\cdot}10 \times 10^4$	99
Opianic acid	1′–10′	$0{\cdot}04 \times 10^4$	< 1
Hydrastal	1, 3–11	$5{\cdot}16 \times 10^4$	100
6-Vinylpiperonylic acid	1, 3–11	$5{\cdot}02 \times 10^4$	97
6-Bromo-α-hydroxy-homopiperonyl bromide	3–11	$3{\cdot}20 \times 10^4$	62
Benzoic acid	1	$2{\cdot}00 \times 10^4$	39
6-Bromopiperonylic acid	4–11	$0{\cdot}14 \times 10^4$	< 3

The most interesting feature of these results, however, is the unequal labelling of carbon atoms 1 and 3 of hydrastine (relative activity 39 and 60 respectively), indicating differential utilization of radioactive, but not of chemical, precursor for the two segments of the alkaloid molecule. Such a possibility has never been considered, and it has hitherto always been tacitly assumed that biosynthetic utilization of two units of a single radioactive precursor to form the major portion of an end-product would inevitably be associated with equal labelling in the two segments of the latter. Reported work on papaverine[6] and morphine[9] has tended to support this assumption, which, nevertheless, is not generally valid. Equal incorporation of radioactivity into the two segments of such a 'dimeric' product is obligatory only if the 'doubling' step in the biosynthetic sequence is of the type $2A \rightarrow A'-A'$, that is, if two identical units of 'monomeric' precursor combine to yield a true dimer as a biosynthetic intermediate (direct doubling).

If the 'doubling' step were preceded by one or more dissimilar structural modifications of either or both 'monomer' units, such as $A \rightarrow X$, $A \rightarrow Y$: $X + Y \rightarrow X'-Y'$ (indirect doubling), then the ratio of radioactivities in the two segments of the product will depend on a number of factors. The most important of these are (*a*) the number of metabolic steps in each branch of the sequence prior to 'doubling', (*b*) the rate of each of these steps, (*c*) the size of the metabolic pool of each branch intermediate, (*d*) possible utilization of branch and 'dimeric' intermediates for other metabolic processes (leakage), (*e*) possible feed-in of branch intermediates from other, non-radioactive, precursors, and (*f*) the turn-over rate of the product. Equal incorporation into both segments of the product will be approached in a single-dose feeding experiment at infinite time, provided there is no leakage or feed-in of branch-precursors, no leakage of 'dimeric' intermediates, and provided the product continuously accumulates. Unless these restrictions hold, any ratio of radioactive incorporation in the two segments of the product, including a fortuitous ratio of one, is possible. The ratio will vary continuously with time, and its numerical value in any single experiment will

depend on the length of time allowed to elapse between initial administration of the tracer and harvesting of the plant.

Although the major portions of each of the two segments of hydrastine (atoms 3–10, and 1, 1′–7′ respectively) are now shown to be derived from a tyrosine unit, the two fragments differ in oxygen substitution (atoms 11, and 8′–9′ respectively). In addition, a one-carbon unit (atom 10′) is required to complete the skeleton of the alkaloid. Any or all of these modifications of the tyrosine units may take place either before or after the 'doubling' step.

Work on the variation of the isotope ratio of the segments with time is proceeding and may throw further light on the dynamic aspects of alkaloid biosynthesis in *Hydrastis*. The systematic degradation of the radio-berberine, isolated from the present feeding experiments, will also contribute to the clarification of the problem.

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Ultracentrifugal Differences in Soybean Protein Composition

Soybeans vary widely in size, shape, colour, and chemical composition[1]. Their chemical composition is affected by varietal and environmental factors, and large differences in gross chemical composition are reported. For example, the protein content for 10 varieties over a 5-year period ranged from 36·6 to 53·2 per cent[2].

Little is known concerning the possible variation of the individual components within the protein fraction. The existence of such differences has been suggested on the basis of amino-acid analysis of various soybean protein preparations[3–6], but more direct evidence is desirable. Our results with ultracentrifugal analysis indicate more definitely that some soybean samples differ appreciably in protein composition.

Soybean proteins were extracted at room temperature from 3·0 gm. of dehulled, hexane-defatted flakes by stirring for 30 min. with 100 ml. of potassium phosphate–sodium chloride buffer, *p*H 7·6, $\mu = 0{\cdot}5$, 0·01 *M* with respect to mercaptoethanol[7]. After centrifuging, the insoluble residue was re-extracted twice for 15 min. with 50-ml. portions of the same solvent. The three extracts were combined, concentrated by pervaporation, and equilibrated against the extraction buffer by dialysis prior to ultracentrifugal analysis.

Fig. 1*A* is the ultracentrifugal pattern for the unfractionated proteins of Clark soybeans grown in 1958 near Farmer City, Illinois. Four fractions are present with sedimentation constants of about 2, 7, 11, and 15*S* and the pattern resembles those recorded earlier[7,8]. Two other American varieties grown in Iowa gave similar patterns.

Fig. 1*B* is the ultracentrifuge pattern for the proteins from Hakuhou No. 1 soybeans grown in 1959 at Miyagi Experiment Station, Miyagi, Japan. (We are indebted to T. Watanabe, Food Research Institute, Tokyo, for supplying the samples of Japanese soybeans.) Again four sedimenting fractions are observed, but there are large differences in the percentages of the 7*S* and 11*S* fractions. Also, the 15*S* fraction is reduced in concentration and is less evident as compared with that fraction in the Clark variety. Patterns similar to Fig. 1*B* were obtained for two other soybean varieties grown at Miyagi. The distribution of components shown in Fig. 1*B* was unchanged when proteins from Japanese soybeans were extracted with water and analysed in the extraction buffer. Furthermore, the ratio of 7–11*S* fraction was not altered in the globulin fraction obtained by acidifying an aqueous extract of soybean meal to *p*H 4·5.

Extractability of soybean proteins depends on variety, age of beans, and possibly location of growth[9]. However, the differences between Clark and Hakuhou No. 1 soybeans cannot be due to differential extraction of the 7*S* or 11*S* fractions since more than 95 per cent of the nitrogenous components were extracted from each sample.

Similarity of the three soybean varieties grown in the same location in Japan tentatively suggests that the differences observed in Fig. 1 are caused by environmental factors.

Variations in protein composition have been detected in corn[10], wheat[11], and barley[12] by a number of methods, including isolation of individual fractions, electrophoresis, and ultracentrifugation. So far as we know, the data we report are the first direct evidence that similar variations occur in soybeans. Further

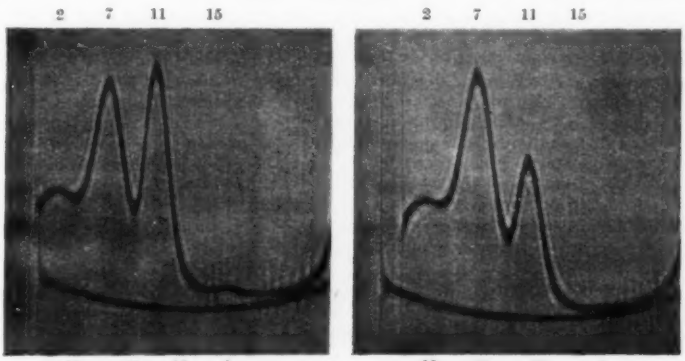

Fig. 1. Ultracentrifugal patterns for (*A*) Clark variety and (*B*) Hakuhou variety soybeans. Numbers across the top of the patterns give sedimentation constants in Svedberg units, across the bottom, the percentage composition. Compositions were determined by area analyses which are corrected for radial dilution. Solutions were analysed in a 30-mm. double-sector cell at 47,660 r.p.m. Protein concentrations ($N \times 6{\cdot}25$) in gm./100 ml. were (*A*) 1·53 and (*B*) 1·24

work is under way with a number of Japanese and American varieties of soybeans to determine the effects of variety and location of growth on protein composition.

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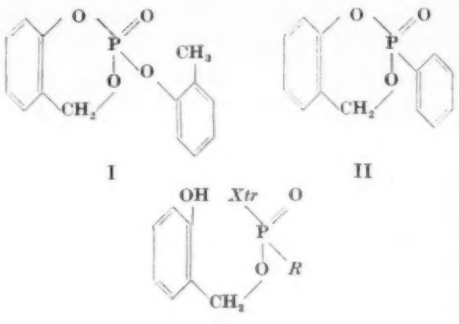
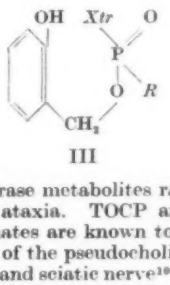
Biological Activity of a Tri-o-Cresyl Phosphate Metabolite

Tri-*o*-cresyl phosphate (TOCP) is metabolized *in vitro* and *in vivo* to form potent esterase inhibitors[1–5]. The nature and biological activity of the metabolites were investigated.

Rats administered radiophosphorus-labelled TOCP were found to excrete radiolabelled di-aryl phosphates, mono-aryl phosphates and phosphoric acid in the urine. Three anti-esterase metabolites were present in the liver, intestine and faeces. Incubation of TOCP with rat liver microsomes fortified with reduced diphosphopyridine nucleotide also yielded three esterase inhibitors of similar chromatographic characteristics to those produced *in vivo*. The major esterase-inhibiting metabolite formed *in vivo* was isolated on silicic acid–'Celite' columns with benzene–ether mixtures. Examination of the infra-red spectrum and hydrolysis products suggested structure I for this metabolite. Reaction of *o*-cresylphosphoryl dichloride with *o*-hydroxybenzyl alcohol yielded 2-(*o*-cresyl)-4H-1,3,2-benzodioxaphosphoran-2-one, b.p. 159–161° C./0·09–0·1 mm. mercury, $n_D^{24.5°} = 1{\cdot}5584$. This compound was identical with the major metabolite in respect to infra-red spectrum, chromatographic characteristics, and anti-esterase activity. One of the other esterase inhibitors appeared to be the *o*-hydroxymethyl derivative of I. The mechanism of esterase inhibition was investigated by reaction of chymotrypsin (*Xtr*) with I and a phenyl phosphonate analogue, II. Both appeared to phosphorylate by opening the cyclic phosphate structure at the P—O— aryl bond to yield III.

An ataxia and demyelination is induced in hens and certain other species by TOCP[6–9]. A similar ataxia in hens resulted from subcutaneous administration of 4–8 mgm./kgm. I, whereas 240 mgm./kgm. II failed to yield ataxia. Preliminary studies indicate a demyelination with I similar to that induced by TOCP. The high activity of I relative to that of TOCP in producing neurotoxicity in hens suggests





III

that the anti-esterase metabolites rather than TOCP *per se* yield the ataxia. TOCP and other ataxia-producing phosphates are known to effect prolonged *in vivo* inhibition of the pseudocholinesterases of hen brain, spinal cord and sciatic nerve[10–13]. The function of this pseudocholinesterase which hydrolyses carboxylic esters of choline is unknown. Experiments with hens established that it also hydrolyses carboxylic esters of thiamine, that is, *O*-acetyl and *O*-propionyl thiamine, an observation consistent with previous reports on the substrate specificity of cholinesterases[14]. The demyelination in hens produced by TOCP is identical with that with certain other organophosphates, and similar lesions appear in hens on a thiamine-deficient diet[8]. Thiamine has been noted as a neuroactive material in peripheral nerves[15] and as a constituent of myelin in peripheral nerves of rabbits[16]. Inhibition of the pseudocholinesterase might result in an accumulation of carboxylic esters of thiamine to impair localized functions of the nerve requiring free thiamine or other thiamine derivatives.

In accordance with this hypothesis, thiamine and other compounds were tested extensively for their effect on the ataxia in hens from TOCP and/or I. Compounds tested alone or in conjunction with thiamine included *O*-acetyl thiamine, tocopherol and cortisone and their acetates, pilocarpine, saponin, and several aldoxime-type cholinesterase reactivators. With varying dosages and multiple administration of these materials before and after the organophosphate, no relief of the neurotoxic symptoms was evident.

TOCP potentiates the toxicity to mammals of malathion (*O,O*-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate)[4,17]. The intraperitoneal LD_{50} for mice was 150 mgm./kgm. for each of I and II. Simultaneous injection of malathion (LD_{50} alone of 1,500 mgm./kgm.) in a 10 : 1 ratio with the cyclic phosphates yielded LD_{50} values of about 1/45th those of either component alone. I and II were similar in this respect, and both yielded marked *in vivo* inhibition of the esterases of plasma and liver hydrolysing the carbethoxy groupings of malathion. The activity of these two compounds in malathion potentiation was greater than that of 120 other di-aryl and tri-aryl phosphates studied which lack the cyclic phosphate structure. The pattern of esterase inhibition in mice resulting from TOCP administration[4] also appeared with I, although the latter acted much more quickly and at a much lower dose.

Metabolic activation occurs with many tri-aryl phosphates containing *o*-methyl, *o*-ethyl, *o*-*n*-propyl and *p*-ethyl groupings[4,5]. *In vivo* hydroxylation of the *o*-methyl and subsequent cyclization to anti-

esterase metabolites appears to account for the biological activity of TOCP and certain other tri-aryl phosphates containing *o*-methyl groupings. A similar mechanism involving α-hydroxylation and cyclization might explain the conversion of *o*-ethyl and *o*-*n*-propyl derivatives to anti-esterases. A different mechanism must be involved with tri-*p*-ethylphenyl phosphate.

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Isolation of Sterol Esters from Human Fæces

THE importance of obtaining more precise information regarding the sterol excretion pattern in human beings has recently become apparent and has been emphasized by Aylward[1]. The normal clinical laboratory techniques for stools, namely extraction of dried material or wet extraction coupled with saponification make impossible the isolation of any sterol esters which may be present. This difficulty in isolating (as distinct from estimating) sterol esters was indeed common to all lipid extracts until the methods developed by Börgstrom[2], and others, were applied by Fillerup and Mead[3] to extracts from animal tissues. Using silicic acid, they were able for the first time to separate cholesterol esters from triglycerides. We have applied a modification of this technique to the lipids of human fæces and have been able to isolate cholesterol ester fractions; and, using gas-liquid chromatography, have examined the fatty acids present in the esters.

Because the amount of sterol esters in human fæces is very small, it was necessary to extract large quantities of material. Accordingly, collections from five volunteers were arranged for periods of 6–9 days, and the extraction procedure was commenced immediately on the receipt of each sample. Preliminary experiments indicated that fæcal pigments interfered with the chromatographic separations, and it was therefore decided to extract the stools first with 0·9 per cent saline, a procedure which removes some of the bacterial lipids[4]. The solid obtained by centrifugation was then extracted successively by ethanol, ether and petroleum ether; precautions were taken to minimize oxidation by carrying out the procedures, wherever possible, under oxygen-free nitrogen.

Table 1. COMPONENTS OF PETROLEUM ETHER-SOLUBLE FRACTION FROM HUMAN FÆCES, CHROMATOGRAPHED ON SILICIC ACID (ref. 5)

Fraction	Case 1	Case 2	Case 3	Case 4	Case 5
Hydrocarbons and pigments	8·8	5·2	10·8	Nil	6·8
Sterol esters	3·5	7·1	2·0	1·0	13·0
Triglycerides	2·1	7·1	2·0	0·6	3·4
Sterols	77·1	76·7	65·4	93·6	62·0
Diglycerides, monoglycerides, phospholipids and pigment	8·5	3·9	19·8	4·8	14·7

In order to increase the proportion of sterol ester in the petroleum ether fraction to be chromatographed, the free fatty acids were first removed by washing with 1 per cent sodium carbonate solution; the extract remaining was dissolved in the minimum amount of hexane and fractionated on a column of Mallinckrodt silicic acid, the ratio of adsorbent: extract being kept at about 100 : 1·5. Pre-treatment of the silicic acid and the elution system followed Barron and Hanahan[5]. Positive oxygen-free nitrogen pressure was maintained, 25 ml. fractions were collected, and elution of the sterol esters was judged to be complete when a negative Liebermann–Burchard test was given. The solvent from the fractions was removed at 40° C. by means of a vacuum oven, the fractions were weighed, and the residues were dissolved in petroleum ether and combined according to the peaks shown when the weighed residues were graphed.

Neither free fatty acid nor free sterol was present in the sterol ester fraction, and the weight of the fraction agreed with the estimations of sterol ester made on the original petroleum ether-soluble fraction by a colorimetric method. Table 1 shows the amount of sterol esters isolated by this method, calculated as a percentage of the total material recovered.

The acids obtained by saponification of the sterol esters from extracts 1, 2 and 5 were methylated and submitted to gas-liquid chromatography, diethylene glycol succinate, ethylene glycol adipate, silicone oil, and dimethylchlorosilane being the stationary phases. The fatty acids present in the extract in the unesterified form, and those from the sterol ester fraction, were markedly similar in type and distribution and ranged from C12–C20 +, saturated and unsaturated.

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Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture

Autoradiographic work in various laboratories on synthesis of nucleic acids in the intact cell has indicated that nuclear RNA is a precursor for cytoplasmic RNA, although different authors have placed different emphasis on the role of the nucleolus and chromatin portion[1-5]. It was suggested that RNA mediates incorporation of pyrimidine nucleotides into DNA, probably through an intermediate fraction[7,8]. In testing and extending these observations, the uptake of tritiated cytidine, uridine, thymidine and deoxyuridine into the various nucleotide fractions was examined for dependence on RNA-ase and DNA-ase, added to the cells in culture before, and at the same time as, exposure to the tritiated precursors.

Tritiated cytidine (specific activity 1 c./m.mole) and tritiated uridine (specific activity 0·62 c./m.mole) were used as common precursors for RNA and DNA at a concentration of 2 μc./ml. of medium. Tritiated thymidine (specific activity 1·9 c./m.mole) and tritiated deoxyuridine (specific activity 4·0 c./m.mole) as specific precursors for DNA at a concentration of 0·4 μc./ml. of medium. RNA-ase was obtained from the Nutritional Biochemicals Corporation in crystalline form, and DNA-ase from the California Corporation for Biochemical Research. Crystalline albumin was obtained from Armour Laboratories, and bovine serum from the Colorado Serum Co.

Leighton tube cultures of HeLa-S_3 cells, either with or without coverslips, containing about equal numbers of cells in the log phase of growth were used. Simultaneously with the addition of tritiated thymidine, or 90 min. before addition of tritiated thymidine, tritiated cytidine, tritiated deoxyuridine and tritiated uridine, the standard culture medium was changed to one supplemented with RNA-ase or DNA-ase (to make a final 0·1, 0·2 or 1 per cent solution), or one containing equivalent amounts of protein in the form of albumin or bovine serum, or one without supplement. The cultures in the various media were incubated at 37° for various lengths of time. At the end of the incubation period, the cells were washed, fixed and extracted differentially with perchloric acid to obtain the different nucleotide fractions (acid-soluble pool, RNA and DNA), or they were fixed with methanol and prepared for autoradiography with or without prior histochemical extraction of RNA with perchloric acid. Details of the methods used have been described in refs. 6 and 7.

Table 1 lists the total tritium activity of the separated nucleotide fractions, soluble pool, RNA and DNA, after 10 min. exposure to the tritiated precursors. The results show that the incorporation of tritiated thymidine and deoxyuridine is depressed in the HeLa cell by RNA-ase and to a smaller extent by DNA-ase. Tritiated cytidine and uridine incorporation into DNA is not inhibited and its incorporation into RNA is slightly enhanced by RNA-ase, in agreement with Ledoux's data on ascites tumour cells[9].

Similar results were obtained autoradiographically (grain counts) and will be reported later. The cells showed morphological changes when 0·2 per cent to 1 per cent RNA-ase was used, as a decrease in basophilic staining property in the cytoplasm as well as in the nucleoli, which often were distorted. The decreased basophilia might be explained in part by a complex formation of RNA with RNA-ase, thus preventing the basic stain from binding[10].

Incorporation of tritiated thymidine and tritiated deoxyuridine into the 'soluble pool' was inhibited by RNA-ase. Tritiated thymidine added to the cells simultaneously with the medium containing RNA-ase was more readily incorporated than when tritiated thymidine was given at 90 min. after addition of the medium containing RNA-ase to the cells. This in addition seems to indicate that the RNA-ase acts not primarily on the cell membrane but intracellularly[10]. The various effects reported for RNA-ase on cells might all be explained by an effect on RNA and not on enzymes, even if they are associated with microsomes[11], nor on energy-delivering reactions[10]. The RNA-ase used at present was tested in the fixed HeLa cell, and was shown to remove selectively RNA, leaving DNA intact.

If one assumes specificity of RNA-ase for RNA also in the living HeLa cell and despite the lack of further results on reactions in the soluble fraction, the inhibition of uptake of thymidine and deoxyuridine by RNA-ase in this work suggests that incorporation of those precursors into the nucleic acids in the HeLa cell may be mediated by, or depend on,

Table 1. Total Tritium Activity of Nucleotide Fractions (Soluble Fraction, RNA and DNA) in counts/min. and as percentage of Control, after 10 min. Exposure of the Cells to Different Tritiated Pyrimidine Nucleosides in Regular Culture Medium and in Medium containing RNA-ase ,or DNA-ase, or Equivalent Amounts of Protein as Bovine Serum or Albumin. Results are Averages from Duplicate Experiments

Precursor and time	Culture medium	Soluble pool (counts/min.)	Soluble pool Percentage control	RNA (counts/min.)	RNA Percentage control	DNA (counts/min.)	DNA Percentage control
Tritiated thymidine 90 min. after medium change	Control	468	100	—	—	1,053	100
	+ RNA-ase (0·2 per cent)	171	37	—	—	262	25
	+ DNA-ase (0·2 per cent)	317	68	—	—	594	56
Tritiated thymidine 90 min. after medium change	Control	1,836	100	—	—	4,718	100
	+ albumin	1,670	91	—	—	4,412	94
	+ serum	1,356	74	—	—	3,736	79
	+ RNA-ase (1·0 per cent)	189	10	—	—	408	9
Tritiated thymidine with medium change	Control	1,399	100	—	—	3,378	100
	+ albumin	1,266	90	—	—	2,449	72
	+ serum	1,278	91	—	—	2,520	75
	+ RNA-ase (1·0 per cent)	844	60	—	—	1,621	48
Tritiated deoxyuridine 90 min. after medium change	Control	210	100	17	100	110	100
	+ RNA-ase (0·2 per cent)	130	62	10	59	17	15
	+ DNA-ase (0·2 per cent)	92	44	15	88	51	46
Tritiated cytidine 90 min. after medium change	Control	27,771	100	14,172	100	526	100
	+ RNA-ase (0·2 per cent)	28,920	104	18,311	129	554	105
	+ DNA-ase (0·2 per cent)	30,131	108	14,830	105	476	90
Tritiated uridine 90 min. after medium change	Control	25,579	100	5,885	100	305	100
	+ RNA-ase (0·2 per cent)	29,623	116	8,046	137	334	109
	+ DNA-ase (0·2 per cent)	22,306	87	6,201	105	350	115

an RNA, probably in the soluble pool. RNA synthesis *de novo* apparently would not be dependent on this same RNA fraction, since tritiated cytidine and tritiated uridine incorporation was not depressed.

Since the labelling of DNA from tritiated cytidine and tritiated uridine was only slightly altered by RNA-ase and DNA-ase, the depression of the incorporation of tritiated thymidine and tritiated deoxyuridine apparently does not reflect a depression of the rate of DNA-synthesis.

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Effect of Boric Acid on the Phosphoglucomutase of Pea Seeds

It has been suggested that a possible role of boron in higher plants is to activate the processes involved in the uptake and movement of sugars, and the manner of operation has been attributed to the facility with which boric acid forms complexes with the *cis*-hydroxyl groups of the sugar molecule. Dugger and Humphrey[1] suggest, as the result of assay of a comprehensive range of enzymes from pea seedlings, that boron inhibits sucrose synthesis from uridinetriphosphate, glucose-1-phosphate and fructose, and stabilizes the level of uridine diphosphoglucose as well as increasing the rate of its formation. They consider that boron inhibits starch phosphorylase, thus increasing the availability of glucose-1-phosphate for the formation of uridine diphosphoglucose. The transfer of the former through the normal glycolytic cycle is considered to be unaffected on the basis of assay of specific enzymes, including phosphoglucomutase. The phosphoglucomutase used was apparently prepared from muscle, and, in fact, it has been shown that low concentrations of boric acid specifically inhibit the phosphoglucomutase of pea seeds[2]. The enzyme can be purified by alcohol fractionation at −5° C. and obtained free of phosphorylase and phosphatases which utilize the substrate and products. The phosphoglucomutase activity is always associated with that of phosphohexoisomerase, but this enzyme has been found to be unaffected by boric acid. Fig. 1 shows the extent of the inhibition by a range of concentration in a preparation of this type. The fall in inorganic phosphorus + phosphorus released by hydrolysis at 100° C. in 1 *N* hydrochloric acid (free + 7′P) is used as a measure of formation of glucose-6-phosphate. When the purified system is used, no increase in inorganic phosphorus occurs during the time required for the system to reach equilibrium, and at this time chromatographic assay indicates the presence of only glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate.

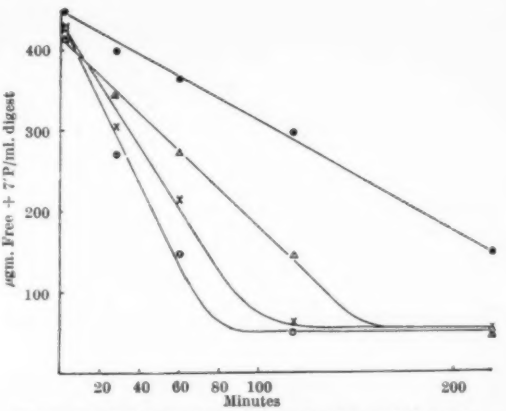

Fig. 1. Effect of boric acid on the activity of a purified phosphoglucomutase preparation from pea seeds. Digest components: glucose-1-phosphate, 13 μmoles/ml., magnesium chloride, 5 μmoles/ml.; glyoxaline/hydrochloric acid buffer *p*H 7·2, 20 μmoles/ml.; enzyme, 0·06 mgm. nitrogen/ml. Concentration of boric acid: 2×10^{-3} *M* (●); 5×10^{-4} *M* (Δ); 2×10^{-4} *M* (×); none (○)

The inhibition of phosphoglucomutase may be complex: for example, Fig. 2*a* shows that there may be an initial lag period, followed by an increase in rate though the final rate is still less than that in the control digests. This type of inhibition can best be explained on the assumption that the inhibitor is rendered ineffective by combination with one of the

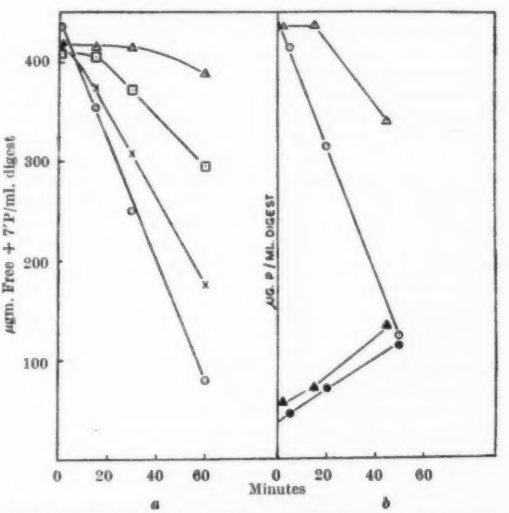

Fig. 2 (*a*) Effect of boric acid on the initial stage of utilization of glucose-1-phosphate by a purified phosphoglucomutase preparation. Main digest components as in Fig. 1. Concentration of boric acid: 5×10^{-3} *M* (Δ); 1×10^{-3} *M* (□); $2{\cdot}5 \times 10^{-4}$ *M* (*X*); none (○). (*b*) Effect of boric acid on the utilization of glucose-1-phosphate by a crude extract of pea seeds (50 gm. dry seeds soaked and extracted with 100-ml. ice-cold 0·005 *M* neutral cysteine). Main digest components as in Fig. 1, enzyme extracts diluted four-fold. Concentration of boric acid: 1×10^{-3} *M* (▲ and Δ); none (● and ○). Open symbols (free + 7′P); filled symbols (free P)

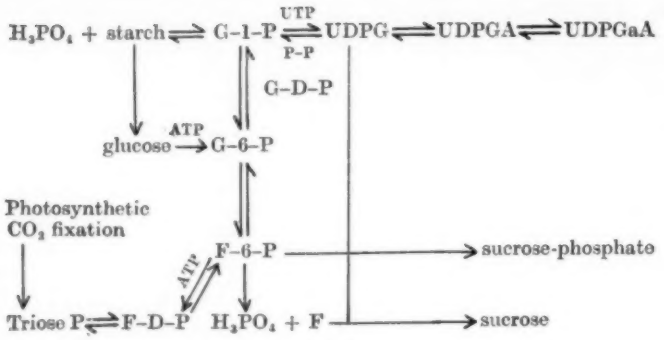

Fig. 3

products of the reaction. This enzyme system involves a low concentration of glucose-1,6-diphosphate as an essential component of the reaction, and on the basis of existing knowledge of the mechanism of action of the muscle enzyme it is likely that phosphorylation of the enzyme occurs; it is at this stage that boric acid possibly has its effect. The crude enzyme is also inhibited by boric acid under conditions in which the contaminating phosphorylase is unaffected as measured by increase in colour with iodine, and release of inorganic phosphate (Fig. 2*b*). The partially purified plant preparations contain appreciable quantities of inorganic orthophosphate as well as bound ketose, and the importance of these components is still under investigation.

The phosphoglucomutase activity is greatest at about *p*H 7·8, but similar percentage inhibitions occur with 0·002 *M* boric acid over a *p*H range of 5·6–8·0. At the *p*H normally used, namely, 7·2, 99 per cent of the added borate would exist as boric acid.

From these results it follows that if utilization of starch is blocked at the phosphoglucomutase stage, two immediate consequences are apparent:

(*a*) The formation of glucose-6-phosphate and hence fructose-6-phosphate and fructose can be prevented by boric acid, thus reducing the amount of fructose-6-phosphate and fructose available for the synthesis of sucrose. Since combined fructose is not normally stored in amounts comparable with glucose polysaccharides, it is reasonable to suppose that fructose arises primarily via the phosphorylated form by the normal glycolytic use of starch.

(*b*) More glucose-1-phosphate is available for synthesis of uridine diphosphoglucose, and in the absence of fructose acceptor it would either accumulate or be available for synthesis of other materials, for example galacturonic acid residues of the cell wall.

At this stage it would be premature to speculate further on the role of boron in higher plants. However, glucose-1-phosphate clearly occupies a key position in the utilization of hexoses, and the scheme of Fig. 3 indicates how the inhibition of phosphoglucomutase by boric acid could be used as a means of regulating closely the diversion of glucose-1-phosphate for different synthetic purposes. It is evident that the mechanism of action of the phosphoglucomutase of pea seeds is similar to that of the muscle enzyme which is unaffected by boric acid[1], so it is unlikely that the inhibiting action is brought about by the formation of complexes with one of the substrates. It is more reasonable to attribute the specific effect on the plant enzyme to the structure of the enzyme protein. The complex nature of the mechanism of action coupled with the type of inhibition shown in Fig. 2 makes kinetic studies difficult, and the elucidation of the type of inhibition involved is obviously necessary before an assessment of the importance of boric acid as a regulatory substance can be critically evaluated.

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PHYSIOLOGY

Number and Arrangement of Osteons in Corresponding Regions of Homotypic Long Bones

Tetracyclines administered *in vivo* become incorporated into recently laid down rapidly calcifying bone-tissue; the labelled material can be detected by its fluorescence in ultra-violet light[1–3]. This technique represents a very valuable new tool in the investigation of bone growth and reconstruction.

The problem investigated here is whether it occurs, and what is the extent of structural symmetry, in various sectors and levels of the shaft cortex of homonymous long bones in the right and left side, in other words, in portions of osseous tissue which are largely submitted to equal mechanical stresses and undergo a series of parallel changes during bone growth and remodelling. In the present communication, results are reported on the distribution and number of new-forming Haversian systems (secondary or replacement osteons). This work is introductory to a study of the factors which govern bone

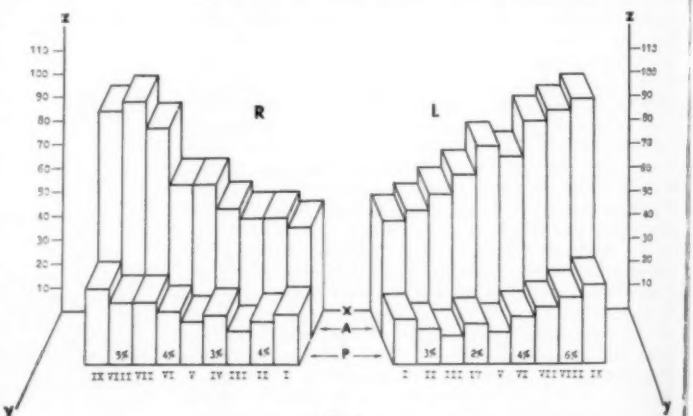

Fig. 1

reconstruction, and has also a practical meaning since the structure of the 'controlateral' bone is generally taken as the normal test of the bone submitted to experimental procedures.

Young growing and adult dogs were given *per os* demethylchlortetracycline ('Ledermycin') 30–40 mgm./kgm. body-weight for 3 days and killed 7 days later. Cross-sections of the entire diaphysis were taken from various long bones of both sides; care was taken to cut out the sections exactly at the same shaft-levels in each couple of homonymous bones. Generally, 5–15 planoparallel cross-sections 1 mm. apart, starting from the middle level of the diaphysis, were serially sawn and ground to a uniform thickness of about 60μ. Photomicrographs were made of each section in ultra-violet light (Ortholux Leitz microscope).

The distribution and number of the osteons labelled by a fluorescent ring were analysed in the photomicrographs of the shaft cross-sections, divided into four 90° quadrants. A rather close symmetrical correspondence in the arrangement of the newly formed osteons resulted in each of the four 90° quadrants of each couple of sections (right and left) at the same level. The 90° quadrants were further bisected and the distribution of the osteons analysed in each 45° sector of the left and right sections. Each 90° quadrant was divided also into an outer and an inner band by means of a line equidistant from the periosteal and the endosteal surfaces of the cortex. When comparing such sectors and zones in the right and left bone, a fairly close symmetry in the distribution of new-formed osteons was still apparent; such a correspondence was no longer found when comparing, on both sides, small areas of the sectors or zones.

The new-formed labelled osteons were counted in the corresponding 90° quadrants and 45° sectors of the right and left bone. The percentage differences in the number of labelled osteons between the right and left bone were as follows: 90° quadrants, max. 14·6 per cent—min. 0·87 per cent; 45° sectors, max. 28·5 per cent—min. 3·4 per cent. An analogous course in the variation of the number of the osteons was recorded in the corresponding 45° sectors of the two sides when studying the series of successive cross-sections from the middle shaft-level to the metaphysis.

An attempt was also made to assess the amount of bone tissue laid down in the osteons forming in corresponding sectors and levels of the right and left bone shafts during the period of tetracycline treatment. The labelled areas were cut out from enlargements (× 110) on photographic paper of constant known weight and their total weight computed as percentage of the weight of the entire area covered by bone tissue in the 45° sectors of the right and left side after removal from the photographs of the areas occupied by non-osseous material (for example, lumina of vascular canals and resorption cavities).

As an example, the results obtained from the analysis of an 18-mm. cylinder of the right and left tibial shaft in a 6-month-old dog are shown (Fig. 1). The number of labelled osteons in the left (L) and right (R) tibia is given as a function of the level of the section and of the position of the 45° sector. On the x axis are plotted the levels of the shaft cross-section in caudo-cranial order, commencing from the centre of the diaphysis (I, II, III, etc.); on the y axis are plotted A, the anterior, and P, the posterior 45° sectors of the 90° antero-medial quadrant; on the z axis are plotted the number of labelled osteons in each sector of every section. The per cent ratios between the amount of the new-formed labelled osseous tissue and all the bone material of the posterior sector P is reported only for four levels.

Altogether it seems that the differences in the distribution and number of Haversian systems (structures of the second order) forming during a given period of time in strictly corresponding regions of compacta of homotypic bones are not much more conspicuous than the differences in the shape and thickness of the cortex (structures of the first order) in the same regions.

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Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices

Adrenalectomy increases, and cortisone decreases, the incorporation of radioactivity from amino-acids labelled with carbon-14[1,2], and from amino-acid precursors[3], into the protein of isolated rat diaphragm. Implicit in these observations is the possibility that the adrenal steroids alter protein biosynthesis in muscle by an effect on the intracellular protein synthesizing machinery. The experiments to be described are part of an investigation of that possibility. Because of the relative ease with which myocardium is homogenized and fractionated that tissue was selected (in preference to skeletal muscle) for this work.

Adrenalectomy increased, and cortisone depressed, the incorporation of amino-acids into myocardial slices in a way, qualitatively and quantitatively, similar to their effects on skeletal muscle[1,3] (Table 1).

There is no reason to believe, then, that there is a fundamental difference in the effect of adrenal cortical hormone on protein biosynthesis in cardiac as compared with skeletal muscle, and myocardium would appear to be a suitable tissue in which to investigate the mechanism of the effect of corticosteroids on protein synthesis.

In an attempt to identify the locus at which corticosteroids influence protein biosynthesis, heart

Table 1. Effect of Adrenalectomy and Hydrocortisone on Incorporation of ^{14}C-Phenylalanine into Protein of Myocardial Slices

Experimental condition	Radioactivity incorporated into protein (counts/min./mgm.)
Intact	111 ± 9·5
Adrenalectomized	159 ± 6·7
Adrenalectomized, hydrocortisone, 0·4 mgm./day	123 ± 7·0
Intact, hydrocortisone, 4 mgm./day	77 ± 5·1

Male Sprague–Dawley rats of 200–300 gm. were used; adrenalectomy and hormone treatment were carried out as described before (refs. 1 and 3). Rats were decapitated, the hearts excised and sliced freehand on a cold glass surface; endocardial slices were discarded. Approximately 100–200 mgm. of tissue was incubated with 2 ml. Krebs–Henseleit bicarbonate medium, 1·5 mgm. glucose and 0·3 μc. 3-^{14}C-DL-phenylalanine (0·23 mM). Incubation was for 2 hr. at 37° C. in a Dubnoff shaker (152 cycles/min.); the gas phase was 95 per cent oxygen–5 per cent carbon dioxide. At the end of the incubation period the protein was isolated and its radioactivity determined (ref. 4). Each figure is the mean ± *S.E.* of the mean of six observations. The effect of adrenalectomy ($P < 0·001$) and of 4 mgm./day of hydrocortisone ($P < 0·01$) are significant.

Table 2. Distribution of Radioactivity from ^{14}C-Phenylalanine in Protein of Cell Fractions from Myocardial Slices: Effect of Adrenalectomy and Cortisone (2 mgm./day)

Fraction	Condition	Radioactivity incorporated into protein (counts/min./mgm.) 0 min.	5 min.	15 min.	30 min.	60 min.
Slice	*I*	0	10	18	28	51
	A	0	8	28	53	97
	C	0	6	16	23	39
Cell debris	*I*	0	8	16	21	48
	A	0	10	24	46	79
	C	0	6	10	19	33
Mitochondria	*I*	1	5	15	31	40
	A	0	7	36	75	150
	C	1	6	12	28	32
Microsomes	*I*	0	8	23	45	80
	A	0	85	92	210	452
	C	0	6	13	28	70
Soluble	*I*	0	6	25	46	105
	A	0	17	54	116	242
	C	0	5	18	39	75

Approximately 500–700 mgm. of myocardial slices were incubated, for varying periods of time, in 5 ml. of Krebs–Henseleit medium containing 1·5 mgm./ml. of glucose and 0·1 μc./ml. of L-phenylalanine-U-^{14}C (0·01 m*M*). After incubation, two slices were removed for separate analysis and the remainder of the sample was fractionated at 0–4° by a modification of the method used by Zamecnik and Keller for liver (ref. 5). The tissue was minced in 0·35 *M* sucrose containing 0·01 *M* unlabelled DL-phenylalanine, homogenized at 1,200 r.p.m. for 10–15 sec. with a loose-fitting pestle (clearance 0·014–0·019 cm.) and for 20–30 sec. with a tight-fitting pestle (clearance 0·010–0·015 cm.). The homogenate was spun for 10 min. at 700*g* in a Servall *SS*-1 centrifuge. The supernatant was decanted and spun at 15,000*g* in a Spinco model *L* preparative ultracentrifuge for 30 min.; the 15,000*g* supernatant was decanted and spun again at 105,000*g*. The final soluble fraction (supernatant) was retained. The pellets obtained at 15,000 and 105,000*g* were rinsed and drained. Four fractions were obtained in this way: (1) at 700*g* a pellet consisting of unbroken cells, nuclei and myofibrils (hereafter referred to as 'debris'); (2) at 15,000*g* a fraction referred to as mitochondrial, although it is likely some small non-respiratory granules are also present; (3) at 105,000*g* the microsomes, and (4) a final soluble or supernatant fraction. Each figure is the average of two determinations. *I*, Intact; *A*, adrenalectomized; *C*, cortisone-treated.

slices were incubated with ^{14}C-phenylalanine and the distribution of radioactivity in the protein of cell fractions determined. Since the distribution of radioactivity was measured at a number of different time-intervals for intact, adrenalectomized and cortisone-treated animals (Table 2), of practical necessity, only duplicate observations could be made. For this reason the results must be considered to be preliminary.

After adrenalectomy the specific radioactivity of the protein in each of the fractions of heart muscle (debris, mitochondria, microsomes, soluble fraction) is increased (Table 2). The increase, after 1 hr. of incubation, is by far the greatest in the microsomal fraction (448 per cent as compared with 90 per cent in the slice itself). The magnitude of the effect of adrenalectomy on amino-acid incorporation into heart microsomes suggests the possibility that they are the intracellular site at which adrenalectomy acts to enhance protein biosynthesis. This would be analogous to the finding by Korner[6] that it is the liver microsomes, rather than the other participants in intracellular protein biosynthesis, which are stimulated by adrenalectomy. This suggestion—there is no direct evidence for an effect of hormones on microsomes in muscle—needs testing in an *in vitro* system.

Cortisone depressed amino-acid incorporation into each of the cellular fractions of myocardium. In contrast with the changes after adrenalectomy the decrease after cortisone was equal in each fraction (20–30 per cent), and not nearly so great. The distribution of radioactivity in the protein of the several fractions, then, offers no clue to the mode of intracellular action of cortisone in depressing protein biosynthesis; if anything, it suggests the most important effect of cortisone is not intracellular. On this score, there is evidence[7] that cortisone depresses amino-acid penetration into the muscle cell interior, an effect that accounts, at least in part, for the depression of protein biosynthesis; this effect need not be reflected in a selective depression of amino-acid incorporation into any specific cell fraction.

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Action of Plasmin on Cartilage Matrix *in vivo*

Intravenous injection of unactivated papain into young rabbits leads to collapse of their ears due to loss of matrix[1], reduction of the chondroitin sulphate in the ear cartilage[2,3] and the appearance of chondroitin sulphate in the blood and urine[3,4]. Activated papain does not produce this gross effect, and it has been suggested[5,6] that the active enzyme combines with inhibitors in the blood, whereas the inactive papain is activated by sulphydryl groups in cartilage itself and attacks the mucopolysaccharide/protein complex, releasing the chondroitin sulphate.

Lack and Rogers[7] reported the liberation of chondroitin sulphate from cartilage *in vitro* by the blood protease, plasmin. They suggested that the rapid chondrolysis which occurs in joint infections by streptococci and staphylococci might be due to kinases produced by these organisms activating plasminogen in the surrounding fluid. Astrup *et al.*[8] have shown that a diffusable activator of plasminogen is present in synovial membrane and articular cartilage and have postulated that this provides the mechanism for the removal of fibrin from joint cavities after trauma or hæmorrhage.

It now appears that if sufficient plasminogen reaches cartilage *in vivo* there is a sequence similar to that observed with papain. The cartilage matrix is depleted, chondroitin sulphate diffuses out and is afterwards excreted in the urine. As loss of matrix may be demonstrated following immersion of cartilage in plasminogen, it is presumed that the enzyme is activated *in situ* by tissue activator.

In the experiments summarized here we have injected rabbits with crude papain, rabbit plasminogen and human plasmin respectively. We also exposed ears, screened by asbestos from the rest of the body, to 10 min. infra-red radiation, giving 10 min. exposure to each side of the ear. The crude papain was provided by Messrs. A. M. Zimmerman (London), human plasmin by the Blood Products Division, Medical Research Council, and rabbit plasminogen was extracted from fresh rabbit serum by Kline's method[9]. Urines were collected in twenty-four hour periods and stored at −10° until assayed. Their content of acid mucopolysaccharide was measured by the method of Di Ferrante and Rich[10], the results being expressed in terms of the glucuronic acid content of the precipitated acid mucopolysaccharide fraction, as estimated by the method of Dische. Urine was collected for two days prior to injection and afterwards until the glucuronic acid content returned to the pre-injection level. The results obtained are summarized in Table 1.

Table 1. EXCRETION OF ACID MUCOPOLYSACCHARIDES IN RABBIT URINE

No.	Weight of rabbit (kgm.)	Treatment	Acid mucopolysaccharide excretion (mgm. glucuronic acid/24 hr.)		No. of days after treatment to reach excretion peak
			Pre-treatment level	Post-treatment peak	
1	1·5	Papain (3 ml., 2 per cent)	2·2	4·3	1
2	1·3	Rabbit plasminogen	1·3	3·0	5
3	0·8	Rabbit plasminogen	2·3	6·8	3
4	1·4	Rabbit plasminogen + local $CHCl_3$	1·5	4·3	1
5	1·5	Human plasmin	1·5	3·3	1
6	1·0	Infra-red to 1 ear	2·0	3·5	4
7	1·0	Infra-red to 2 ears	1·4	8·0	4

The ears were closely observed during these experiments and it was noted that papain produced greater collapse and also a greater tendency to hæmorrhage around the veins. A rise in the excretion of chondroitin sulphate was not always accompanied by a collapse of the ears: sometimes there was no more than a softening or curling of the tips.

We postulate that plasminogen, diffusing from the blood, is activated in cartilage and attacks the protein/chondroitin sulphate complex in a manner similar to papain; it appears that this may be achieved by increasing local vascular permeability, which allows plasminogen already in the blood to pass through to the matrix.

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Cholinergic Transmission in Fish Muscle

IT is known that the enzymes needed for the synthesis and the destruction of acetylcholine are present in fish muscle in large amounts[1,2], and that certain electric organs, probably derived from muscle, are sensitive to acetylcholine[3]. It is therefore usually taken for granted that neuromuscular transmission in fish occurs by a cholinergic mechanism, as in other vertebrates, but there is, in fact, little evidence that fish muscle can be activated by acetylcholine, or that acetylcholine is normally released by motor-nerve-endings in fish.

Although muscle from the goldfish (*Cyprinus auratus*) is not markedly sensitive to acetylcholine injected into the bathing solution, acetylcholine applied from a micro-pipette can have a powerful depolarizing action. The membrane potential of fibres in the deep part of the lateral muscle, at the level of the visceral cavity, was recorded with a conventional glass micro-pipette filled with 3*M* potassium chloride, and acetylcholine was released from a similar micro-pipette filled with 3*M* acetylcholine chloride (Roche Products). When the acetylcholine micro-pipette was brought close to sensitive spots on the surface of the fibre, rapid depolarization could be produced by permitting acetylcholine to diffuse out of the tip; if sufficient acetylcholine was released, the fibre contracted. The effect was best seen by passing short pulses of outward current through the pipette. The resulting potential changes (Fig. 1) resembled normal junctional potentials observed in the same muscle during repetitive stimulation, but their time-courses were much slower (about ten times slower in the case of the potential shown in Fig. 1). The very sensitive regions were extremely sharply localized, as in other vertebrate muscle, and the maximum sensitivity was comparable with that of frog muscle (that is, about 1 mV. depolarization per *n* coulomb of charge delivered through the acetylcholine pipette). Similar observations were made on the deep lateral muscle of catfish (*Ameiurus nebulosus*).

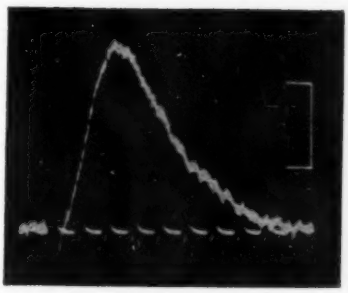

Fig. 1. Acetylcholine potential produced by a 1 msec. (3 *n* coulomb) pulse of outward current, recorded in deep lateral muscle of goldfish. Vertical scale: 1 mV.; horizontal scale, 10 msec.

Indirect evidence of acetylcholine-release in fish muscle is provided by the susceptibility of junctional potentials to the blocking action of tubocurarine (10^{-5} w/v) or to potentiation by eserine (10^{-5} w/v). In Fig. 2, eserine is shown to have caused a great increase in the amplitude of spontaneous miniature potentials recorded in a fibre of the deep lateral muscle with an intracellular micro-pipette.

Fig. 2. Spontaneous potentials recorded in a fibre of deep lateral muscle of goldfish: (*A*) control; (*B*) 8 min. after adding eserine sulphate (10^{-5} w/v). Vertical scale, 1 mV.; horizontal scale, 1 msec.

These observations seem to give strong presumptive evidence of cholinergic transmission in at least some fish muscle.

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Removal of Milk Constituents from the Mammary Gland of the Ewe

A number of experiments was conducted on the ewe to determine the role of lymph draining from the lactating mammary gland in the resolution of the milk constituents. Lymph flowed continuously for many weeks from a polyethylene catheter inserted into the main lymphatic duct draining the mammary gland. Initial investigations were concerned with the changes in composition of the lymph when the lamb was removed from its mother early in lactation. Plasma and lymph samples were collected at intervals for 7–14 days and the concentrations of free fatty acids, volatile fatty acids, cholesterol, phospholipid, total esterified fatty acids, protein, potassium, calcium and inorganic phosphate were measured. In addition, attempts were made to determine the presence of milk proteins in the lymph by the technique of specific precipitation in agar[1] and also by electrophoresis on cellulose acetate.

In three sheep out of six, the lymph, normally clear, became distinctly milky in appearance when the udder was very distended with milk. The milkiness persisted for as long as 2 days in one of the sheep and for only 4 hr. in another. The milky lymph contained increased concentrations of total esterified fatty acids, calcium, inorganic phosphate and potassium, and microscopic examination of the lymph under dark-ground illumination revealed the presence of particulate fat. Protein peculiar to milk was also identified in the milky lymph by the specific precipitation technique (Fig. 1). This could not be demonstrated electrophoretically. There was no evidence for the presence of this protein, nor significant change in the constituents measured in lymph which was not milky in character. More recent experiments demonstrated that a large proportion of human serum albumin containing iodine-131, which had been introduced into the gland sinus, was removed intact by way of the lymphatics. Under conditions in which the gland became very distended with milk, the radioactivity in the lymph rose to a high level and then decreased exponentially (Fig. 2). Lower levels of radio-activity were found in the lymph draining from mammary glands which did not become very distended with milk.

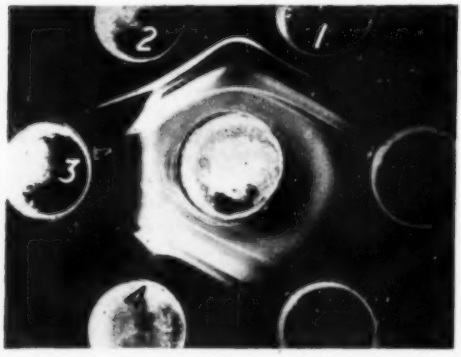

Fig. 1. Lines of precipitation between the central well containing ewe skim milk antiserum and the surrounding wells containing (1) non-milky lymph, (2) milky lymph, (3) diluted milk, and (4) wether's serum. A broad line of precipitation can be seen opposite the well containing milk. This line crosses all the precipitation lines opposite the well containing wether's serum and represents the precipitation between specific antibodies and protein in milk not found in plasma. This broad milk protein line is continuous with a line (often seen divided) opposite the well containing the milky lymph. No such line was found opposite the well containing the non-milky lymph

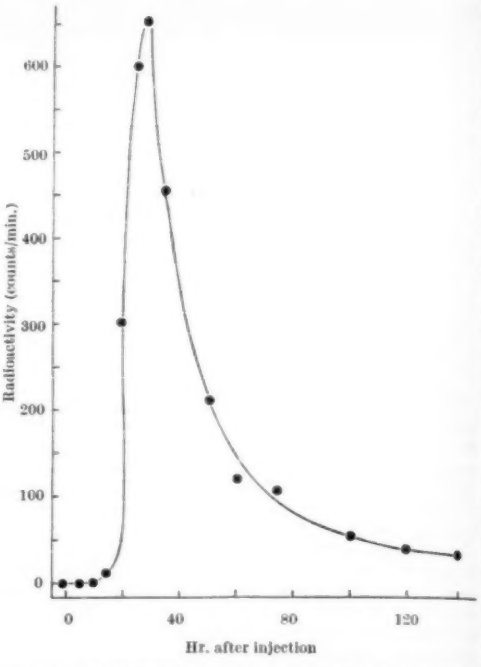

Fig. 2. Appearance of radioactivity in the lymph draining from a mammary gland following the introduction of human serum albumin labelled with iodine-131. Since the radioactivity was found in the trichloracetic acid precipitate it is reasonable to assume that the human serum albumin is being removed intact

These results indicate that fat and proteins of milk may be removed by the lymphatics of the mammary gland. The escape of milk from the gland when the udder is grossly engorged may be associated with the actual rupture of some acini or milk ducts. The finding that the lymph became rapidly milky following the insertion of a hypodermic needle deep into the mammary tissue may be significant in this regard. However, it appears that much of the secreted milk in the udder is removed by less-obvious means. Heyndrickx and Peeters[2], in their studies on the constituents of mammary lymph collected from freshly slaughtered cattle, point out that the lymph collected from the udder which had not been milked out prior to slaughter was excessively turbid, and analysis of this lymph showed that it contained milk constituents. The apparent ease of escape of milk into the lymph under these conditions may be associated with tissue anoxia or it may be a character-

istic feature of the dairy cow and related to the higher rate of milk production.

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HÆMATOLOGY

Recovery and *in vivo* Survival of Rabbit Erythrocytes

Human and other mammalian red cells have been frozen and thawed with limited hæmolysis using a variety of cooling conditions and protective additives. Slow cooling in high concentrations (30 per cent) of glycerol[1], moderately rapid cooling in plasma containing sugars[2], and ultra-rapid cooling in low concentrations (6 per cent) of glucose[3] have allowed high recoveries (90–95 per cent) of red cells of which 80–90 per cent survive at least 24 hr. when transfused.

Polymeric solutes, including polyvinylpyrrolidone (PVP), dextran, polyethylene glycols, and albumin, afford effective protection for red cells as measured by *in vitro* recoveries[4]. Under conditions of rapid cooling and warming, polymers are the most efficient of all protective agents[5]. To our knowledge, however, only one investigator has reported *in vivo* survival studies on red cells frozen in the presence of polymers. Sloviter[6] observed poor viability (20 per cent) of rabbit and human red cells frozen with polyethylene glycols unless thawed cells were washed free of the polymer. Washing, however, lysed some 80 per cent of the initially recovered cells.

We have observed, in work described in this report, that rabbit blood containing a low concentration of PVP, a neutral, hydrophilic polymer, can be rapidly frozen and thawed with erythrocyte recoveries of greater than 90 per cent. Thawed blood was transfused directly, without removal of the polymer, and the red cells exhibited a 24-hr. survival averaging 93 per cent. Table 1 shows results obtained in four experiments using 24 separate blood specimens.

Rabbit blood was collected in acid-citrate glucose (3 vols. blood, 1 vol. ACD). Polyvinylpyrrolidone of average molecular weight 40,000 (*K*-30 PVP, Oxford Laboratories, San Francisco) in saline was added (4 vols. blood-ACD, 1 vol. PVP) to give a final concentration of 7 per cent in the mixture. Twenty to forty ml. of the mixtures was frozen rapidly in capped aluminium tubes of 17–33 mm. diameter by mechanical shaking in liquid nitrogen. After storage at − 170° C. for several days, samples were thawed by mechanical shaking in water at 45° C.

Recoveries of red cells were determined using hæmatocrits and colorimetric measurements of supernatant and total hæmoglobin as oxyhæmoglobin in 1 : 100 dilutions in aqueous sodium carbonate.

Aliquots of thawed blood were labelled with chromium-51 (ref. 7), (Abbott Laboratories, sterile sodium 'Rachromate') and an accurately measured volume was infused through an ear vein. Total infused radioactivity as red cells was measured on a separate aliquot. All chromium-51 measurements were made by scintillation counting using a Nuclear Chicago *DS5*–5 well counter. Blood samples were taken from the opposite ear into oxalate at 30–60 min. and again at 24 hr. after infusion. The percentage survival of red cells at each sample time was calculated using apparent red cell volumes previously determined on each rabbit by auto-transfusion of labelled unfrozen blood.

Table 1. Recovery and Survival of Red Cells in Rabbit Blood containing Polyvinylpyrrolidone

Exp.	No. of samples	Container diameter (mm.)	Red cell recovery (per cent)	Red cell survival (per cent) 30–60 min.	24 hr.
1	6	17	92 ± 3	110 ± 15	98 ± 12
2	6	17	87 ± 6	97 ± 4	98 ± 6
3	6	17	93 ± 1	93 ± 16	89 ± 17
4	6	33	96 ± 1	97 ± 7	85 ± 7

In experiment 4, samples also were taken at 2–3 day intervals up to 12 days. Percentage survival at each sample time was corrected for labelled cells removed by previous sampling and chromium elution. Identical measurements were made on six additional rabbits which received unfrozen blood. Frozen cells were lost more rapidly during the first 48 hr.; subsequent rates of loss of frozen and control cells were equal (about 1·8 per cent per day). Approximately 85 per cent of the transfused frozen cells exhibited normal *in vivo* viability.

Results indicate that polyvinylpyrrolidone, long studied as a plasma expander, may prove useful as a protective agent in the low-temperature preservation of whole blood suitable for direct transfusion after thawing.

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PATHOLOGY

An Iso-Antigen (γB^A) of Mouse γ-Globulin Present in Inbred Strains

Hereditary iso-antigens (allotypes) of rabbit γ-globulin were discovered in 1956 by Oudin[1], and independently in 1958 by Dubiski *et al.*[2]. Since then many publications have appeared reporting the serological, genetical and immunochemical characteristics of rabbit γ-globulins[3–6]. More recently, antigenically different types of serum globulins have been described in guinea pig[7], man[8] and baboon[9].

In the present experiments, two inbred strains (*BALB/c* and *C57BL*) of mice were used; the *BALB/c* mice were first immunized, by Dubiski's method[2], with *Proteus vulgaris* (*X* 19). *Proteus* cells coated with anti-*Proteus* sera were afterwards injected subcutaneously twice a week (15 times) into individuals of the *C57BL* strain. In this way, five pairs of mice were immunized with five individual sera (more particularly the γ-globulin selected by bacteria) of

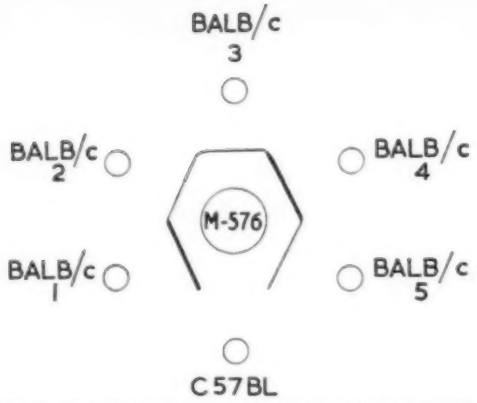

Fig. 1. Precipitation in 1·5 per cent agar gel between a *C57BL* mouse antiserum (*M*-576, in the central well) and sera of six different mice

the *BALB/c* strain. Each serum was therefore used for immunization of two mice. The animals were bled ten days after the last injection.

We tested the immune sera in 1·5 per cent agar gel against the individual *BALB/c* sera used for injections. Only six of ten mice produced precipitating antibodies. They showed an identity reaction varying only in strength. An example of this reaction is illustrated in Fig. 1.

Immunoelectrophoretic analysis showed that the antigen involved in the precipitation is mouse γ-globulin (Fig. 2).

For further investigations, the four strongest antisera were chosen and sera of several mouse strains tested. All four antisera reacted with all sera of individual mice of the strains: *BALB/c*, *C3H/He* and 'Champagne-Glaxo' (Table 1). None of the antisera was precipitated with serum of any *C57BL* mouse, in which strain the antibodies were produced. The experiments showed clearly that the *C57BL* mice lack a γ-globulin antigen, which we will call γB^A, present in each of the other strains of mice tested.

Snell[10] injected *BALB/c* mice with the sperm of *C57* mice and demonstrated an antigen which was absent in the *BALB/c* strain. S. Dubiski (unpublished results) found that rabbit γ-globulin antigens were present not only in the serum but also in the seminal plasma. It seems possible that the antigenic determinants shown by Snell and those described by us are common to serum γ-globulin and sperm and perhaps also seminal plasma.

Since the antisera produced in all *C57BL* mice reacted with sera of every mouse of the other strains, it would be possible in future to use pooled antisera

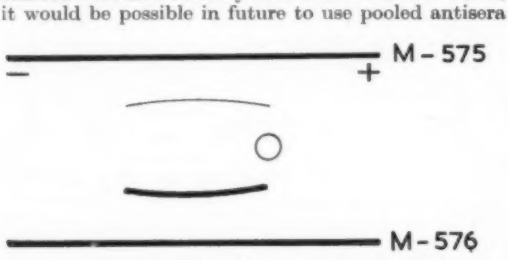

Fig. 2. Immunoelectrophoresis in 1·5 per cent agar gel of a *BALB/c* mouse serum (barbiturate buffer pH 8·6, 0·06 *M*, 10 V./cm. for 1 hr.). The antisera in the troughs were produced in *C57BL* mice

Table 1. Precipitation Tests of Mouse Anti-Mouse Sera

Mouse antisera (*C57BL* anti-*BALB/c*)	Mouse sera as antigens							
	C57BL		*BALB/c*		*C3H/He*		'Champagne-Glaxo'	
	+	−	+	−	+	−	+	−
M-571	0	25	25	0	25	0	20	0
M-572	0	25	25	0	25	0	20	0
M-575	0	25	25	0	25	0	20	0
M-576	0	25	25	0	25	0	20	0

The figures indicate the numbers of sera tested.
+, indicates a positive reaction; −, indicates a negative reaction.

to test a large number of mice belonging to different strains. Further immunization between various strains of mice is necessary to find how many hereditary γ-globulin antigens are present, and which of them are shared by particular strains. It would also be interesting to know if different γ-globulin antigens exist within an inbred strain.

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Cell Transplants of Avian Lymphomatosis treated with Hydrogen Peroxide

Leukosis is considered to be the most serious disease problem in chickens to-day. After Holman[1] reported his success in destroying implants of Walker 256 adenocarcinoma by replacing rats' drinking water with a dilute solution of hydrogen peroxide, it was decided to see if such treatment would alter the course of leukosis in chickens.

Fleischmann *et al.*[2] reported that survival-time of implanted rats was not lengthened by hydrogen peroxide treatment and hydrogen peroxide did not decrease the size of tumours induced by X-irradiation. Paoletti *et al.*[3] were unable to influence the growth of Ehrlich adenocarcinoma and Walker 256 adenocarcinoma in hydrogen peroxide-treated rats. Ghadially and Wiseman[4] found no difference in survival-time between rats treated with hydrogen peroxide and untreated rats which had been implanted with *RD* 3 sarcoma or Walker adenocarcinoma. Makins and Tanaka[5] prolonged the life of rats with ascites sarcoma to 20 days in contrast to 10 days for controls by intraperitoneal injection of 1 ml. of 2 per cent hydrogen peroxide. Holleroft *et al.*[6] increased the effect of X-irradiation on regression of transplanted lymphosarcoma by intravenous injection of hydrogen peroxide immediately before X-irradiation, but hydrogen peroxide administered 5 min. before

or after X-irradiation did not affect regression. Sugiura[7] was not able to demonstrate any favourable effect on transplanted tumours when the animals drank hydrogen peroxide but there was a moderate inhibitory effect on sarcoma 180 ascites tumour when large doses of hydrogen peroxide were given daily. Jeffree[8] reported no effect on survival-time between hydrogen peroxide-treated and untreated rats. Choraży *et al.*[9] reported no significant increase in survival-time between hydrogen peroxide-treated and untreated animals.

Approximately 4 million *RPL* 12 lymphomatosis tumour cells prepared and counted according to the method described by Mohos and Kidd[11] were injected intramuscularly into 20-day-old Leghorn cockerels. Three per cent hydrogen peroxide was given directly into the crop in one experiment. In the other, the chickens were given a 0·45 per cent solution in drinking water. All birds were kept in batteries and observed daily.

Seventy-six chickens were treated 3 times daily, beginning on the day of implantation, with 1 ml. of 3 per cent hydrogen peroxide directly into the crop. Fifty-two per cent died with lymphomatosis compared with 44 per cent deaths in the control group. A χ^2 test of significance between the treated and check groups resulted in a χ^2 value of 0·658, d.f. = 1, $P = 0{\cdot}40$–$0{\cdot}50$. The difference was not significant.

Nineteen chickens were not treated until tumours were readily palpable, after which time a 2-ml. dose was given 3 times daily. From the same group, 6 others were given 5 ml. 3 times daily. From the group given 2 ml., 10·5 per cent died, and from the 6, 50 per cent died. In the control group, 64 per cent of 25 died. A χ^2 test for differences between the 2 ml. dose and check group resulted in a χ^2 value of 10·761, d.f. = 1, $P = 0{\cdot}001$.

The test indicated the need for further investigation of the effect of hydrogen peroxide. Therefore, a second experiment was initiated in which the dosage and method used by Holman[1] were followed. Hydrogen peroxide at 0·45 per cent level was given continuously in the drinking water to 78 chickens from the day of inoculation until nearly all were dead from tumours. In 78 others, treatment was started 5 days after inoculation when tumours were first palpable. Eighty-two controls were not treated. In the first treated group, 87·5 per cent died with tumours and in the second group, 83·3 per cent died. In the controls, 91·4 per cent died: $\chi^2 = 2{\cdot}33$, d.f. = 2, $P = 0{\cdot}30$–$0{\cdot}40$.

Since treatment of the transplanted tumours in chickens was not encouraging, it was decided to see if hydrogen peroxide would destroy the cells by direct contact. Portions of tumours were macerated in a Ten Broeck grinder and mixed with 3 parts of 3 per cent hydrogen peroxide. Chickens were injected with 0·2 ml.; tumours developed and destroyed most of the chickens. Tumours were taken from chickens which had been treated with all dosage-levels of hydrogen peroxide and transplanted into other birds. All tumours grew readily and killed the chickens.

In neither instance had hydrogen peroxide altered the activity of the tumour in chickens. Tumours from treated chickens were examined histologically, but there was no difference between tumour cells from treated and untreated chickens.

Hydrogen peroxide was not effective in prolonging the life of chickens implanted with lymphomatosis tumour cells. In the one possible exception where only 10·5 per cent of 19 did die, the possibility of host-resistance cannot be ruled out since some chickens are known to have more resistance than others to this disease. Furthermore, the small number of birds in this group may not have been adequate for a good test.

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IMMUNOLOGY

Serum Transferrins in the Genus *Macaca*: Species Distribution of Nineteen Phenotypes

In the genus *Macaca*, rhesus monkeys show a remarkable polymorphism of transferrin, the iron-binding protein, whereas cynomolgus monkeys (the Philippines variety) show only one molecular form of the protein[1,2]. Two-dimensional starch-gel immunoelectrophoresis[3] was used in previous work[1,2] to determine (with the aid of a uni-specific chicken anti-human transferrin serum) the number of transferrin components in the macaque sera. Then comparative one-dimensional starch-gel electrophoresis in the *tris* discontinuous buffer system was used to establish the subtle differences in the mobilities of transferrin, and fifteen phenotypes[2] due to various combinations of eight molecular forms of the protein were detected among rhesus monkeys. These results have now been confirmed by determinations based on the iron-binding property of transferrin. We have also adopted a provisional letter system of nomenclature which has simplified identification of the macaque transferrin phenotypes, thereby making it easier to discover new phenotypes. In this connexion a re-examination of the rhesus sera and an examination of sera from additional macaque species have now revealed nineteen transferrin phenotypes, sixteen being present in the rhesus group alone.

Fig. 1 presents the results of an experiment which involved: addition of iron-59 to rhesus sera representing the fifteen previously discovered phenotypes, analysis of these sera by vertical starch-gel electrophoresis in borate buffer[4], and then demonstration of the iron-binding components by autoradiography[5]. Again the fifteen phenotypes due to the different combinations of the eight molecular transferrins were observed. Each molecular form was assigned a letter, *A* designating the transferrin of slowest mobility and *H* that of fastest mobility. (The mobility increases from *A* to *H* in paper electrophoresis as well as in starch-gel electrophoresis and in the *tris* buffer as well as in the borate buffer.) In lettering the phenotypes it was assumed that when the transferrin genes were homozygous a double dose of a single molecular form of transferrin would be synthesized; but, when heterozygous, single doses of at least two molecular forms

Table 1. Species Distribution of Nineteen Transferrin Phenotypes in the Genus *Macaca*

	No. of sera examined belonging to:																			
	AD	*BC*	*BD*	*B(D)G*	*BH*	*CC*	*CD*	*CE*	*CF*	*CG*	*CH*	*DD*	*DF*	*DG*	*FF*	*FG*	*GG*	H^1H^1	*HH*	Total
M. mulatta	2	2	1	1	1	*19*	12	6	2	5	5	*9*	4	4			*3*		1	77
M. irus												*95*								95
M. nemestrina							1			1		*4*	2	4	*1*	1	*3*			17
M. speciosa																		*10*		10

Note: The number of sera in each homozygous phenotype is in italics.

would be synthesized. Thus the phenotypes *CC*, *DD*, *GG*, and *HH* are thought to be controlled by homozygous combinations of genes and the other phenotypes by heterozygous combinations. As can be observed in the figure, transferrin *C*, *D*, *G* or *H* has a larger serum concentration in a 'homozygous' phenotype than in a 'heterozygous' phenotype.

The previous suggestion that in the genus *Macaca* there are sharp species differences in the degree of polymorphism of transferrin and in the frequencies of the transferrins[1] is substantiated by the results summarized in Tables 1 and 2. Sixteen phenotypes,

Table 2. Frequency of Molecular Transferrins

	Percentage of sera examined possessing:								
	A	*B*	*C*	*D*	*E*	*F*	*G*	H^1	*H*
M. mulatta	2·6	6·5	66·3	42·9	7·8	7·0	16·9		9·9
M. irus				100·0					
M. nemestrina			11·8	64·7		23·5	52·9		
M. speciosa								100·0	

four homozygous and twelve heterozygous, have now been recognized in rhesus monkeys (*M. mulatta*) from the Nepal border area of India (77 specimens from three different shipments). One of these phenotypes, a rare one, *BD*, is a new discovery. The transferrin having the highest frequency was *C*. In contrast to the extensive transferrin polymorphism of the rhesus monkeys, only the *D* transferrin has been found in cynomolgus monkeys (*M. irus*) from the Philippines (ninety-five specimens from four different shipments). The pig-tailed macaques (*M. nemestrina*) are again a polymorphic species. Although only seventeen specimens were examined, eight phenotypes (three homozygous and five heterozygous) were observed. Two of these phenotypes, *FF* and *FG*, are new discoveries. The most common phenotype in the rhesus group, *CC*, was not seen in the pig-tailed group. The *D* transferrin had the highest frequency in this group. The stump-tailed macaques (*M. speciosa*), like the Philippines cynomolgus monkeys, may prove to be transferrin homozygotes. The ten specimens examined contained only the H^1 transferrin. This transferrin, another new discovery, was not seen in the other species. H^1 moves faster in electrophoresis than *G*, but not quite as fast as *H*. The physico-chemical nature of the differences in the macaque transferrins and the evolutionary basis for the selection of the phenotypes have yet to be elucidated. In any attempt to do so, it would seem important to investigate the possible role of ecological and geographical factors on the distribution of the transferrin alleles in the genus *Macaca*. In this connexion, *Macaca speciosa* inhabits central India, which is supposed to be quite high in iron content.

We wish to thank Mr. W. Carey Parker for determining the iron-binding components of the rhesus sera by autoradiography and for suggesting the letter system of nomenclature for the rhesus transferrins. We also gratefully acknowledge the valuable help of Dr. P. E. Ayers of the Parke-Dale Unit, Parke Davis and Co., in providing the rhesus and cynomolgus sera, and Dr. R. C. Wolf of the Primate Laboratory, University of Wisconsin, in providing the pig-tailed macaque sera. The stump-tailed macaques, obtained from the Ferndale Pet Shop, were originally shipped from Calcutta, but were captured in central India. A collection of primate sera from Dr. John Buettner-Janusch is now under examination. His collection contains *Macara irus* specimens from Malaya. These specimens, in contrast to those from the Philippines, do show a transferrin polymorphism. This work is being supported by a grant (*G*–14152) from the National Science Foundation.

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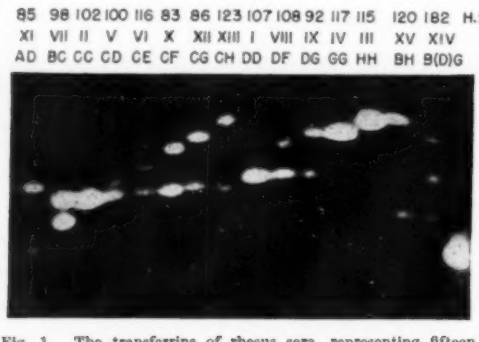
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XI VII II V VI X XII XIII I VIII IX IV III XV XIV
AD BC CC CD CE CF CG CH DD DF DG GG HH BH B(D)G

Fig. 1. The transferrins of rhesus sera, representing fifteen phenotypes, are compared in vertical starch-gel electrophoresis with the transferrin of normal human serum (far right). Note that the rhesus transferrins migrate faster than the human transferrin. At the top of the figure are the sample designations of the rhesus sera. Many of these samples were used in the original demonstration of rhesus transferrin phenotypes (ref. 1). The Roman numerals designate the phenotypes by the previous nomenclature (refs. 1 and 2). The letters, symbolizing the molecular transferrins, designate these phenotypes by the new nomenclature

Products of Mild Acid and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances

The preparations of *M*- and *N*-blood group substances obtained by phenol extraction of human erythrocyte stroma are mucoids[1]. They contain, on an average, 10 per cent of hexoses, 6·5 per cent of hexosamines, 1 per cent of fucose, 17 per cent of *N*-acetylneuraminic acid and 7·5 per cent of total nitrogen[1,2].

In an attempt to learn more about the carbohydrate structure of these mucoids, the products of their hydrolysis were studied. The conditions of hydrolysis were similar to those applied to bovine sub-maxillary gland mucoprotein by Gottschalk[3].

Acid hydrolysis: A solution of 15 mgm. of *M*- or *N*-substance in 2 ml. of 0·05 *N* sulphuric acid was heated at 80° for 40 min., afterwards cooled and neutralized with saturated barium hydroxide solution to *p*H 6. Barium sulphate sediment was centrifuged

and the supernatant was dialysed exhaustively against distilled water at 2°. The dialysate was lyophilized.

The presence of *N*-acetylneuraminic acid in the dialysate was proved by characteristic reactions (diphenylamine and 2-thiobarbituric acid), paper chromatography in three solvent systems (I: *n*-butanol/*n*-propanol/0·1 *N* hydrochloric acid, 1: 2: 1 v/v; II: *n*-butanol/pyridine/water 6: 4: 3 v/v; III: pyridine/ethyl acetate/acetic acid/water, 5: 5: 1: 3 v/v) and by conversion into 2-carboxypyrrole[3]. After acid hydrolysis of *M*- and *N*-substances, 85–95 per cent of *N*-acetylneuraminic acid was split off and their group activity disappeared completely. In this process also small amounts of dialysable carbohydrate–amino-acid fragments were liberated.

Alkaline hydrolysis: 60 mgm. of *M*- or *N*-substance dissolved in 10 ml. of water was heated rapidly to 80° and saturated barium hydroxide solution was added to a final concentration of 0·02 *N*. The mixture was kept at 80° for 15 min., then cooled and acidified with 1 *N* sulphuric acid to *p*H 6. A further procedure was the same as that after acid hydrolysis.

By means of paper chromatographical analysis and characteristic reactions the following components were found in the dialysate: *N*-acetylneuraminic acid, small amounts of chromogen I, traces of *N*-acetylhexosamine, galactose (in some preparations) and two unknown compounds: C_1 and C_2. The last-named were separated from the other dialysable components by paper chromatography followed by elution of suitable strips of chromatograms; for the separation of C_1, the dialysate was subjected to chromatography in solvent system I, and for the isolation of C_2, in solvent system II.

Compound 1 (C_1): this was homogeneous in the three solvent systems used, gave a distinct colour with benzidine reagent, and a weak colour with direct Ehrlich reagent for sialic acids. Its R_F value was lower than that of galactose. On treatment with acid (0·05 *N* sulphuric acid, 80°, 40 min.) C_1 was split off into *N*-acetylneuraminic acid and galactose (chromatographical evidence). The presence of *N*-acetylneuraminic acid in C_1 was also stated by the methods described here (see acid hydrolysis). The secondary cysteine reaction of hexoses confirmed the presence of galactose in C_1. The amounts of *N*-acetylneuraminic acid and galactose were almost equimolecular.

C_1 was decomposed into *N*-acetylneuraminic acid and galactose after the action of influenza virus strain Lee at 37°.

Compound 2 (C_2): this was submitted to paper chromatography in two solvent systems: II and III gave one spot visible by direct Ehrlich reagent for sialic acids and by direct Ehrlich reagent in the cold (characteristic for chromogens which are formed on mild alkali treatment of *N*-acetylhexosamines[4]) and moved a little faster than *N*-acetylneuraminic acid. C_2 hydrolysed by 0·05 *N* sulphuric acid at 80° for 40 min., yielded only *N*-acetylneuraminic acid; the chromogen part of C_2 was destroyed during this hydrolysis. Chromogen I formed from *N*-acetylglucosamine was also fully destroyed under the same acid conditions. Moreover, *N*-acetylneuraminic acid in C_2 was identified by methods used here.

After the incubation of C_2 with influenza virus Lee at 37° *N*-acetylneuraminic acid and chromogen I (R_F value = 0·6 in solvent system II and positive direct Ehrlich reaction in the cold) were found.

Such disaccharides as *N*-acetylneuraminyl-galactose (C_1) and *N*-acetylneuraminyl-chromogen (C_2), obtained by mild alkaline hydrolysis of *M*- or *N*-substance, show that *N*-acetylneuraminic acid may combine with galactose or *N*-acetylhexosamine in this biologically active material. The linkage of *N*-acetylneuraminic acid in both disaccharides is α-ketosidic because it is labile in acid at elevated temperature and susceptible to virus α-neuraminidase. The mild alkaline hydrolysis of *M*- or *N*-substance repeated several times supplied the same products; it is possible, therefore, that these disaccharides form an essential part of the prosthetic group of *M*- and *N*-mucoids.

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BIOLOGY

Thiabendazole: a Highly Effective Anthelmintic for Sheep

IN the past year, reports on several compounds with outstanding anthelmintic activity against the gastro-intestinal trichostrongyles and strongyles of sheep have been published. Among the anthelmintics tested were the organic phosphorus compound 4-tertiary-butyl-2-chlorophenyl methyl-methyl phosphoramidate[1], a pyridine derivative, 2-(β-methoxyethyl) pyridine[2], and one in which an organic phosphorus compound and phenothiazine are stated to act synergistically[3].

Very recently, 2-(4′-thiazolyl)-benzimidazole (thiabendazole) has been reported[4] to have outstanding anthelmintic activity against a wide range of nematode parasites in sheep, cattle and pigs, and some activity against nematodes in dogs.

When thiabendazole was tested in sheep in this Laboratory and in the field in Australia at a dose-rate of 50 mgm./kgm. body-weight, it exhibited a very high degree of anthelmintic activity against *Haemonchus contortus*, *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia curticei*, *Nematodirus* spp., *Oesophagostomum venulosum*, *O. columbianum* and *Chabertia ovina*, but it was not effective against *Trichuris* spp.

In some experiments with penned sheep with a pure infection of either *H. contortus* or *T. colubriformis*, anthelmintic activity was assessed from the results of repeated worm egg counts, but in critical experiments with infections with parasites of the large bowel, the activity was assessed from the ratio of the number of worms passed in the fæces to the number of residual worms found at slaughter a week after dosing. Also, controlled experiments were conducted with penned sheep with naturally acquired mixed infections, an assessment of effectiveness being made by comparing at slaughter the worm counts in treated sheep and in control animals.

In field trials, anthelmintic activity was assessed from results obtained from repeated fæcal examina-

tions, comprising worm egg counts and differentiation of the larvæ recovered from fæcal cultures. In some of the field trials it could be assumed that worm burdens would have been increasing just after the time of treatment, because in control sheep slaughtered at an appropriate time, immature worms were present in large numbers.

The results of all the trials indicated that the worm burdens had been reduced to extremely low levels by a single treatment with thiabendazole, and some treated sheep were found to be completely free from infection when slaughtered. In field trials most of the fæcal examinations made within the two weeks following treatment revealed no eggs, and cultures did not yield any larvæ. In these field trials, it was found that post-treatment egg counts either became constantly negative or remained very low, and it can therefore be assumed that any immature worms present at the time of treatment had also been destroyed.

Results from one controlled trial in penned sheep which harboured mixed infections, shown in Table 1, demonstrate the remarkable anthelmintic activity of the compound.

In one field trial with groups of 50 treated and control lambs, selected at random and held on pastures for fattening, the greater gain in weight in the group treated monthly with thiabendazole showed this drug to be a very effective anthelmintic, and the lambs gained weight at a very high and steady rate. In this trial, 36 per cent of the controls, 44 per cent of the group treated monthly with phenothiazine, and 68 per cent of those treated monthly with thiabendazole attained a weight of 70 lb. or more at 4½ months of age.

In critical trials with *O. columbianum* and *O. venulosum* in Australia, Hebden[5] found that all worms were removed from sheep by treatment. His trials with other species gave results comparable with those recorded in this communication.

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Table 1. Numbers of Adult Worms in Sheep after Treatment with Thiabendazole, and in Control Sheep

Sheep	Abomasum			Small intestine		
	Haemonchus contortus	*Ostertagia* spp.	*Trichostrongylus axei*	*Trichostrongylus* spp.	*Nematodirus* spp.	*Cooperia curticei*
Controls						
1	6,000	500	400	3,000	300	3,600
2	1,000	400	200	2,700	650	1,400
3	12,000	3,000	1,200	4,600	1,200	400
4	10,000	1,100	—	3,000	1,100	700
5	3,060	2,000	170	3,000	1,100	1,000
Treated with thiabendazole						
6	—	—	—	—	—	—
7	—	—	—	—	—	—
8	—	—	—	—	300	—
9	—	—	—	—	—	—
10	—	—	—	—	—	—
11	—	—	—	—	—	—
12	—	—	—	—	—	—
13	—	—	—	80	80	—

— indicates the absence of worms in the aliquot taken for counting; this does not necessarily indicate that no worms were present, but that the total could not have exceeded 50.

Growth Stimulation by High Indole-3-Acetic Acid Concentrations

It is well established that the curve depicting the extension growth of coleoptile sections as a function of indole-3-acetic acid concentration in the surrounding medium (the concentration curve) assumes the shape of an optimum curve. In the case of wheat coleoptile sections the curve ascends at a constant rate until the optimum is reached at approximately 10^{-5} *M* indole-3-acetic acid. The descent of the curve above the optimum is conditioned by the inhibiting effect of higher indole-3-acetic acid concentrations. This clear and distinct picture is found at least when an experimental period lasts 24 hr., while for shorter periods the course of the concentration curve is not so well known.

Foster *et al.*[1] found that the concentration curve is an optimum curve even for short-time experiments. They obtained time curves (curves representing extension growth for different concentrations of indole-3-acetic acid as a function of time), which were straight lines having slope coefficients increasing with indole-3-acetic acid concentrations until 10^{-5} *M*. At higher concentrations they still observed linear curves, but with slope coefficients decreasing with increasing concentration. In later investigations by Cleland[2] it is demonstrated, however, that time curves for different concentrations follow the same course for the first (2–4) hr. of growth. Only after a longer time-interval do the curves separate, showing the usual inhibiting effect of high indole-3-acetic acid concentrations. Working with 10-mm. coleoptile sections (wheat, variety Progress), I could not observe any significant separation of time curves for indole-3-acetic acid up to 10^{-3} *M* over a period of about 3 hr., thus confirming Cleland's results.

To examine more closely the non-appearance of an inhibiting effect of high auxin concentrations during the initial course of the time curves, a special technique was adopted. Attempts were made to let the indole-3-acetic acid act before the actual growth processes commenced. This principle has been introduced by Heyn[3]. The method employed was as follows. During a pre-treatment period (first period) the growth of the coleoptile sections was impeded by either a solution with sufficiently high osmotic pressure (sucrose or mannitol dissolved in McIlvaine's citric acid–phosphate buffer, *p*H 4·6 at 1/100) or by keeping the sections at low temperature (about 3° C.). During the first period treatment with indole-3-acetic

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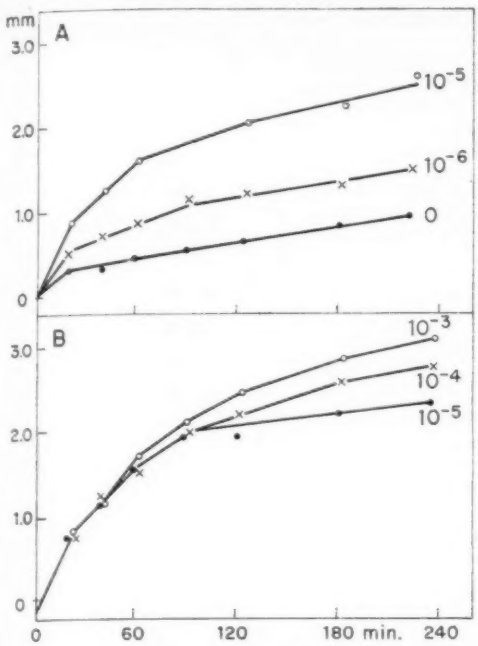

Fig. 1. Growth of wheat coleoptile sections in pure citric acid-phosphate buffer at 23° C. after pretreatment for 2 hr. in growth-impeding sucrose solutions (0·24 *M*) containing indole-3-acetic acid in molar concentrations as shown in the diagrams

acid was carried out with a concentration series ranging from 0–10^{-3} *M*. After a washing with buffer solution to remove any adhering sugar and indole-3-acetic acid the coleoptile sections were transferred to pure buffer and the growth period (second period) commenced. All experiments were carried out at 23° C. except for the pre-treatments performed in the cold.

A 2-hr. pre-treatment period in 0·24 *M* sucrose resulted in a second period of growth as shown in Fig. 1. For practical reasons, only treatment with three concentrations could be included in each experiment (*A* and *B*). The growth increases with increasing indole-3-acetic acid concentrations up to 10^{-3} *M*. Thus there has been a promoting action by high concentrations normally inducing inhibition when applied during the growth period proper. A repetition of the experiment with a 24-hr. growth period showed similar results.

Low temperature in the pre-treatment period had the same qualitative effect as impeding of growth with sucrose. When mannitol was used as osmotic medium the separation of the time curves for high concentrations could not be observed during the first 4 hr. of the second period, but it appeared distinctly after a period of growth of 24 hr. Prolonging sucrose or low-temperature pre-treatment from 2 to 4 hr. did not alter the results—an increase in growth with rising concentrations up to 10^{-3} *M* was still observed. This effect disappeared when the pre-treatment was extended to 17 hr. at low temperature. Some of these experiments showed, however, less growth with 10^{-3} *M* indole-3-acetic acid than with other concentrations. Complete growth cessation could not be achieved with this pre-treatment. Pre-treatment for 17 hr. in sucrose strongly retarded the growth in the second period.

Adding the auxin in the second period following a pre-treatment of 2 hr. in 0·24 *M* sucrose alone resulted after 20 hr. in a concentration curve with an optimum somewhat below 10^{-5} *M* indole-3-acetic acid. Without pre-treatment the optimum was at 10^{-5} *M*. A similar displacement of the optimum was observed by Schneider[4] in experiments with *Avena* coleoptile sections grown in combined sucrose–indole-3-acetic acid media.

Comparison of the concentration curves (20-hr. experiments) for addition of indole-3-acetic acid during the pre-treatment period, and for addition during the growth period, respectively, clearly showed a specific effect of indole-3-acetic acid in the pre-treatment period. The former procedure gave a concentration curve with a distinct minimum between 10^{-6} and 10^{-5} *M* indole-3-acetic acid while the latter showed a growth optimum in the same concentration interval.

A clear interaction has been demonstrated between indole-3-acetic acid added during the first and indole-3-acetic acid added during the second period. The occurrence of this interaction supports Cleland and Bonner's statement[5] that indole-3-acetic acid can be retained in the tissue from the first to the second period and give rise to a residual effect in the latter.

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Agar Disk Method for the Direct Observation of Nematode-trapping Fungi in the Soil

Attempts at the biological control of soil-borne eelworm pests have usually involved a process of green manuring or the addition of some organic amendment to the soil[1–3]. A method has been developed to obtain direct evidence of the effects of such treatments on the predaceous activity of indigenous populations of nematode-trapping fungi in the soil, such work being regarded as fundamental to the investigation of methods using these fungi as agents of biological control.

A weak maize-meal extract agar of the type used for the isolation of predaceous fungi from the soil was used[4]. Disks 1 cm. in diameter and about 3 mm. thick are cut from plates of this medium and placed on microscope slides, four disks usually being arranged in a row on each slide. Each slide is then buried singly in 15-cm. Petri dishes containing soil so that the disks lie about 1 cm. below the soil surface. At weekly intervals the slides are removed and replaced by others bearing fresh disks. On removal from the soil the disk surfaces are washed with a strong, fine

jet of water from a wash-bottle. The disks adhere to the slide and can be examined immediately, unfixed and unstained, under the microscope.

During the decomposition of cabbage leaf tissue, glucose and sucrose, a diversity of nematode-destroying fungi (both trap-forming predators and endozoic parasites) appear in the predaceous phase on the disk surfaces. The disks are in effect used as microquadrats recording to some extent what is happening in that region of the soil in intimate contact with their surfaces.

The soil is used fresh after being passed through a 7-mm. mesh sieve, thus retaining its microflora and microfauna. Its moisture content is held constant throughout the course of any investigation. By varying the type and quantity of amendment added to the soil, and estimating the nematode population each time the disks are examined[5], the behaviour of the fungi under various conditions and their relationship with free-living nematodes can be studied. Usually four replicate dishes, each holding one slide with disks, are used for each treatment used in any experiment.

It is hoped to enlarge on the details of the relationships between nematode-trapping fungi, organic amendments and nematode populations in a later communication. The above work was carried out during the tenure of an Agricultural Research Council studentship.

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Pneumodermopsis paucidens in the North Sea

Clione limacina Phipps is the only gymnosomatous pteropod normally found in the North Sea, but *Pneumodermopsis ciliata* Gegenbaur has been recorded occasionally from Shetland waters[1,2]. In 1960, however, *P. paucidens* Boas was recognized for the first time in the North Sea, where it appeared in collections taken with the small Plankton Indicator[3] and the Continuous Plankton Recorder[4], both sampling in the upper 10 m. It was first recorded in mid-June to the east of the Orkney Islands and, at about the same time, off the north-west coast of Ireland and to the south of Iceland. Thereafter it was found over a wide area (Fig. 1) until early December, with peak numbers of about 10 per m.[3] at the beginning of September. Previously this species has been recorded only from the North Atlantic and off the coast of Ireland[5].

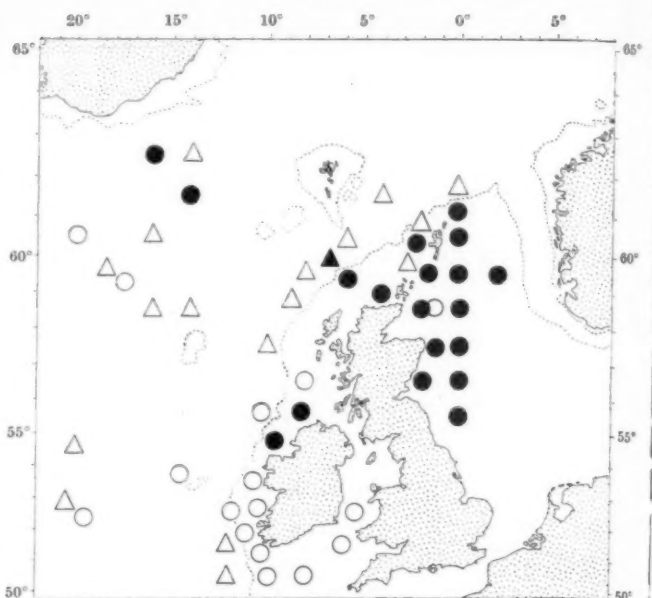

Fig. 1. Chart showing the location of *Pneumodermopsis paucidens* (●) and *P. ciliata* (▲) found in Recorder and Indicator collections in 1960. Previous records from Recorder and Indicator collections in 1956–59, and from the available literature (refs. 1, 2, 6 and 7), are shown as open symbols. Each symbol indicates the centre of a statistical rectangle (1° lat. by 2° long.) in which the species has occurred

Specimens of *Pneumodermopsis* have been re-examined from the collections taken during 1956–59 by Plankton Indicators, in the north-western North Sea, and by Plankton Recorders, throughout the area shown in Fig. 1. Of the total (including those found in 1960) some 700 were *paucidens;* only one of these was taken in the North Sea before 1960. All had the typical radula formula 2:1:2, six hooks and, when they were visible, the characteristic arrangement of the suckers on the median arm. Very few *P. ciliata* were found. These had a radula formula of 6:1:6, many hooks (30–40) and the distal pair of suckers of the median arm had the characteristic "spout at the margin"[6]. Both these species had a range in size of 1–5 mm.

Fig. 1 shows that in Atlantic coastal waters and in the northern North Sea *P. paucidens* was found, whereas in the waters to the west of the continental shelf, both *P. ciliata* and *paucidens* were present.

We are indebted to Dr. J. H. Fraser of the Marine Laboratory, Aberdeen, who confirmed the identification.

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Chemical Character of the Pigments in a new Nitrogen-fixing Micro-organism

Some time ago one of us (Gy. N.) published a communication[1] on a nitrogen-fixing micro-organism from the root nodules of *Lupinus luteus*. By analogy with leghæmoglobin, a red-coloured porphyrin pigment isolated by Virtanen[2] from root nodules of soya beans, Németh presented a working hypothesis of the hæmoglobin-like structure of his red pigment. Interpretation of ultra-violet spectroscopic measurements[1] seemed at the first glance to support this assumption. Furthermore, Matkovics and Tárnoki[3] reported to have isolated—whenever in impure state—a porphyrin methyl ester from root nodules of *Lupinus luteus*. As forecast by Németh[1] more extensive studies were still needed to support this assumption. We wish now to report on our ultra-violet absorption spectral examinations with pigments from strain *Pr-E*/3 of Németh, independently of the work by Schneider, and by Matkovics[5]. On the other hand, paper chromatographic investigation of the acidic extracts prepared from this micro-organism has also been carried out.

In order to separate pigments of different types and structure, before taking up spectra of the strain, *Pr-E*/3 cultivated on a bean agar when air-dry was powdered by quartz sand and, in turn, extracted with pyridine. This sample should contain practically all the pigments as well (Fig. 1, curve 1). Another sample was extracted with ether, the solvent removed very carefully in a nitrogen atmosphere and the residue dissolved in pyridine (Fig. 1, curve 2). A third sample was treated as the second one and then dissolved in *n*-hexane (Fig. 1, curve 3).

The substance which did not dissolve in ether was taken up in pyridine (Fig. 1, curve 4). For the sake of comparison an authentic bovine hæmoglobin dissolved in pyridine (Fig. 2) and paprika carotenoids were investigated spectroscopically in pyridine as well as in *n*-hexane (Fig. 3). Careful measurements of the solutions prepared as given above showed the following spectral results: the ethereal extract showed, when dissolved in pyridine, spectra very characteristic of carotenoids (Fig. 3); no comparison with pyridine-hæmochromogene can be made. The curve of the substance obtained after having been extracted by ether lacks any peak characteristic to carotenoids, but no trace of a pyridine-hæmochromogene could be detected either. The same holds true for the solution of the original pigment in pyridine.

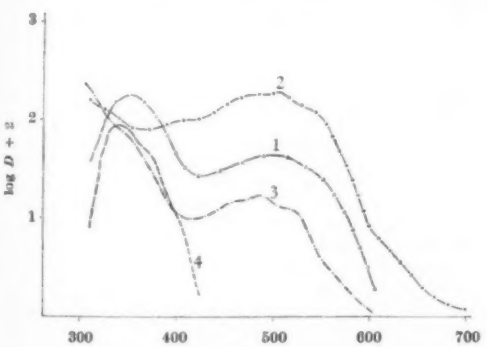

Fig. 1. 1, Strain *E*/3, pyridine extract; 2, strain *E*/3, ether extract, evaporated and dissolved in pyridine; 3, strain *E*/3, ether extract, evaporated and dissolved in *n*-hexane; 4, substance, insoluble in ether, extracted with pyridine

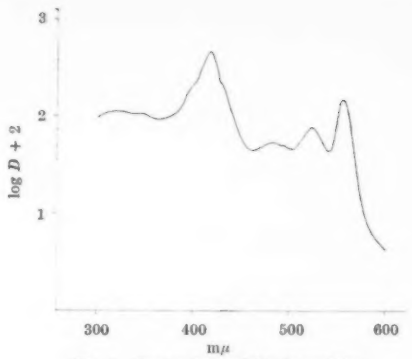

Fig. 2. Bovine hæmoglobin in pyridine

The spectra of the genuine pigment indicate absorption maxima in the range 340–360 mμ (Fig. 4). This can scarcely be ascribed to carotenoids, however; pyridine-hæmochromogene does not absorb in this spectral range either. Accordingly, the chromophore giving rise to this absorption still needs identification. The presence of carotenoids in yeasts is to be expected; for the micro-organism under consideration it has been recorded by others[6].

The red pigment of the strain *Pr-E*/3 could be dissolved quantitatively within a few minutes by distributing by 2 *N* hydrochloric acid and chloroform. The chloroform layer obviously contains fats and carotenoids while the aqueous one proteins of alkaline character and the products of their partial hydrolysis. The aqueous solution has been investigated chromatographically in order to separate amino-acids, porphyrins and flavonoid pigments. Details of this work will be published elsewhere. We shall refer only to the fact that according to the method of Bate-Smith[4] for flavonoids and anthocyanidines with Benedict's reagent a spot characteristic for flavonoids has been found in the case of *E*/3 strain pigment.

In conclusion, the strain *Pr-E*/3 cannot contain considerable amounts of any porphyrin pigments.

The mechanism of nitrogen fixation brought about by these micro-organisms must take quite a different course from that occurring in root nodules of soya beans caused by leghæmoglobin.

Whether carotenoids which are very widespread in micro-organisms, particularly in yeasts, may contribute in any way to nitrogen fixation requires more extensive investigation. Nevertheless, according to

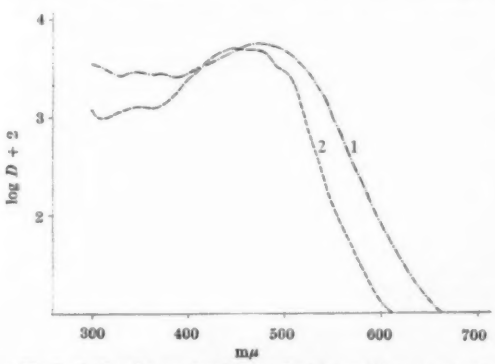

Fig. 3. 1, Paprika carotenoids dissolved in pyridine; 2, paprika carotenoids dissolved in *n*-hexane

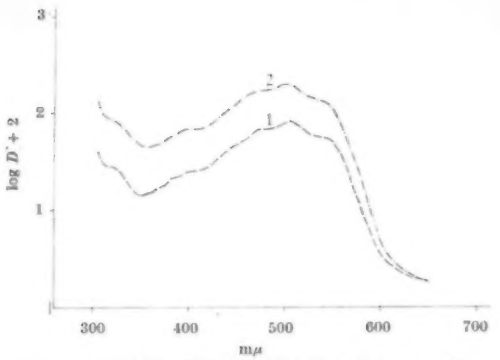

Fig. 4. 1, Pyridine extract of strain *E*/3 raised on a culture medium in distilled water; 2, ether extract of strain *E*/3 raised on a culture medium in distilled water, evaporated, then dissolved in pyridine

Cholnoky *et al.*[5], carotenoids play an important part in oxygen transport in higher plants. It cannot be excluded that oxygen transfer and nitrogen fixation in the yeasts are in some way connected in this case as they are with leghæmoglobin[2].

A more detailed investigation of the pigments of strains *Pr-E*/3 involving flavonoids awaits further investigation.

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Indigotin Content of Woad

Before the general introduction of oriental indigo into Europe in the sixteenth century, woad, the product of the woad plant (*Isatis tinctoria*), was the only commercial source of a blue dye, and its production was the basis of a widespread and important industry. It was the result of a lengthy process involving the crushing of the leaves in specially designed mills, air drying the pulp obtained, grinding the dried material to a fine powder, wetting this, and allowing it to undergo spontaneous fermentation, during which it was kept moist and was frequently turned over. This last process took several weeks to complete, and the material became very hot while the fermentation was active. When cool, the mass was allowed to dry and, after re-grinding and sieving, constituted 'finished woad' ready for the dye-vat. The agricultural operations involved in growing the crop and details of the manufacturing process have been fully described by Hurry[1] and others.

It has long been recognized that the essential dye-stuff of woad was identical with that of indigo derived from various species of *Indigofera*, namely, indigotin, and that the former has a much lower indigotin content than the latter, but no estimate of their relative values based on chemical analysis has ever been made. In the absence of any possibility of obtaining a specimen of the medieval preparation it seemed of interest to grow some of the plant in order to determine its indigotin-yielding capacity and, from this, to arrive at a reasonable approximation to the relation.

In both cases indigotin is derived from a glucoside of indoxyl contained in the leaves. Beijerinck[2] has shown that the glucoside of woad is not identical with that of the species of *Indigofera*, but, since indoxyl results from the hydrolysis of both, the same methods of estimation apply. Methods of manufacture depend, in each case, on the activity of a glucosidase which comes into action when the temperature of the plant material is raised or the normal synthetic activity of the enzyme is reversed by some other means. In the case of indigo manufacture this was brought about by steeping the plant in water at about 90° F. for some hours. The solution of indoxyl thus obtained was then drawn off and oxidized by air, whereby a crude preparation of indigotin was precipitated. In the case of woad the necessary heat was produced in the fermentation process and the indigotin deposited in, or on, the finely divided vegetable material.

The glucosides are readily extracted without change if the operation is conducted in briskly boiling water. Indoxyl may be determined quantitatively in such extracts by hydrolysis with a mineral acid in the presence of an oxidizing agent, followed by sulphonation of the precipitated indigotin and titration of the disulphonate with permanganate. Rawson's ammonium persulphate method, as modified by Bergtheil and Briggs[3], was used for the determination in woad. Plants were grown from seed collected from stock descended in a continuous line from that of one of the few professional growers of woad who was still growing it in England in the early 'thirties of the present century. (Dyeing with woad alone had been abandoned long before this. The survival for so long of the cultivation of the plant on a small scale was due to a demand for it by indigo-dyers, who still used the organic fermentation process to reduce their indigo, since it was thought that its presence in the dye-vat led to the production of richer shades. The amount of indigotin contributed to the vat by the woad must have been negligible relative to that supplied by the indigo, but the finely divided vegetable matter involved doubtless facilitated the fermentation.) Only mature foliage was taken for analysis since neither very young nor very old leaves were used in the industry. Samples were taken at intervals throughout the summer. They showed an average indigotin-yielding potentiality of 0·05 per cent, and very little variation occurred between early July and late September. This is extremely low compared with the poorest *Indigofera* leaf, the analysis of which is recorded (0·54 per cent for *Indigofera sumatrana*). It is noteworthy, however, that in the case of woad there is a much higher proportion of mid-rib in the leaf than in those of indigo plants (roughly 50 per cent in the former as compared with 2 or 3 per cent in the latter) and this contains practically none of the

glucoside. But even if the figure for woad leaf were doubled it would still remain very low by *Indigofera* standards.

Fresh woad leaf contains about 90 per cent water. If we assume that it was completely dry, and that the process of manufacture was fully efficient, the finished product would thus have contained about 0·5 per cent indigotin. The poorest-quality natural indigo which came on the market contained about 30 per cent indigotin, the best some 70 per cent. If we take the mean of these figures as standard we find that finished woad contained only about a hundredth part as much indigotin as average indigo.

It is difficult to understand how, with so little indigotin in the product, dyers were able to produce anything like as dark a shade of blue as we are accustomed to associate with indigo-dyed goods. If they did so, it must have necessitated repeated dippings with intervals for oxidation between them and constant renewals of the dye stocks in the vats. The amount of inert matter involved must have been enormous.

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A Poikilohydrous, Poikilochlorophyllous Angiosperm from Africa

The sedge, *Trilepis pilosa* Boekl, a primary colonizer of rock surfaces on granitic inselbergs in West Africa, has been found to possess the property, most unusual in an angiosperm, of becoming dormant by losing most of its intracellular water during the dry season, with subsequent recovery on moistening. Plants with this property are found mainly among the lower groups (bacteria, fungi, algae, lichens and bryophytes); the word 'poikilohydrous' being first used by Walter[1] in this connexion. Some pteridophytes also exhibit this property: for example, Iljin[2] described drying out and recovery of the fern *Notochlaena marantae* R.Br., and I have found *Selaginella njam-njamensis* Hieron., which colonizes thin soil mats on West African inselbergs, and the epiphytic fern *Platycerium stemaria* (P. Beauv.) Desv., which is sometimes epiphytic on *Trilepis*, to be poikilohydrous.

Richards[3] remarked that the leaves of *Trilepis* last for one season and are green only during the wet months. He also remarked on the rapid development of new green leaves following the early rains. On the rock domes, however, the patches of *Trilepis* are seen to be of various shades of yellow and green following one of the infrequent dry-season showers. With the onset of the rainy season during April, a number of the old, apparently dead leaves nearest the apex of a plant become green. In the laboratory, detached leaves which were so dry as to be brittle and containing approximately 8 per cent water (normal green leaves contain about 56 per cent water), were found to become green and flexible within 24 hr. of moistening under normal conditions of illumination. Similarly the leaves of entire plants became green progressively from the base when the plants were watered; greening did not take place in plants watered and kept in the dark for up to a week, yet it occurred when these were returned to normal daylight conditions.

The colour of the adaxial side of dormant leaves is pale buttercup yellow ($5/_2$) (ref. 4), while the abaxial surface is palest yellow ochre ($o7/_3$). The dormant leaves may be distinguished by this coloration from the buff-coloured dead leaf blades (which are persistent near the stem apices until they decay during the rainy season), and the dead apical regions of the oldest of the dormant leaves. It must be emphasized that the plants appear 'completely dead' in the dry season, as Richards described them; the brittle straw-like texture of the leaves heightens the illusion that they are dead. On moistening, the yellow colours (which are due to carotenoid pigments in the 'chloroplasts') immediately brighten to a deep buttercup yellow ($5/_1$), gradually giving away to green: sulphur yellow ($1/_1$), citron green ($7\ 63/_1$) and fern green ($o8\ 62/_2$) being successive colours recorded for a detached leaf floated on water for thirty hours. At maturity the leaves are a deep green colour, cf. lettuce green (*8* **61**).

Dormant plants of *Trilepis* have been found to retain their viability for at least a year when stored in an air-conditioned room (at ambient temperatures between about 18° C. and 28° C., and relative humidity of about 45 per cent). In the dormant condition the protoplasm is less liable to high- and low-temperature injury, as would be expected from Levitt's[5] summary of work on the limits of high- and low-temperature hardiness. Green *Trilepis* plants rapidly cooled to − 10° C. and kept at this temperature overnight rotted on moistening, while similarly treated dormant plants became green in the normal manner. The lower limits of injury by heat appear to be much higher in the dormant plant than in plants in the hydrated condition; work on this aspect is still in progress. Single leaves will not become dormant—they dry green—and the apical tuft of leaves on a short exised stem may behave likewise: it appears that only with an intact plant or a long portion of stem is dormancy possible under normal conditions. The stem bears a thick 'armour' of dead leaf bases and fibres which loses water rather slowly; this slow drying out of the plant as a whole being possibly connected with the intracellular changes associated with dormancy.

Trilepis leaves have thus been found to last for more than a single rainy season. Their poikilohydrous nature, together with the water-absorbent armour of the stem, enables a rapid start of growth with production of new leaves to be made at the beginning of the wet season (an entire dry plant, in a heavy shower, takes up more than its own weight of water in less than 5 min.). Flowering and production of fruits take place very early in the rains; the nutlet germinates within 24 hr. of moistening, and the young plants thus have a maximum chance of becoming established in their rocky habitat before the onset of the dry season in November.

Trilepis is the second angiosperm found to be poikilohydrous, and closely resembles in its loss and gain of chlorophyll another member of the family Cyperaceæ, *Carex physodes* M. Bieb., described by Vassiljev[6] from the desert flora of Central Asia. Vassiljev mentions the brittleness of the brown, dried-out leaves in *C. physodes* which, as in *Trilepis*, is lost with moistening. The word 'poikilochlorophyllous' is now suggested to describe plants, such as these two angiosperms, which completely lose

and regain their chlorophyll following natural variations in environmental conditions. Poikilohydrous members of the lower groups, including ferns (for example, *P. stemaria*), usually retain their green colour when dormant and may, in contrast, be described as homoiochlorophyllous.

Further work on the ecology of *Trilepis* and other poikilohydrous species from West Africa will be published elsewhere.

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Enzymic Breakdown of Stigmatic Cuticula of Flowers

Enzyme preparations from different micro-organisms are able to break down the leaf cutin of different plants[1,2]. Furthermore, the incompatibility reaction in Cruciferae occurs on the surface of the stigma papillae: pollen tubes are unable to penetrate the cuticle of the stigma cells[3] after self-pollination. Therefore we tried to determine the activity of an enzyme preparation from *Penicillium spinulosum* Thom. on cutin preparations of several sources and especially on stigma cutin isolated from about 10,000 stigmata of black mustard by shaking with pectinase solution following the method in ref. 4, and treatment with ethanol. The results are listed in detail in Table 1. From this it is clear that cutin from

Table 1

Object	Origin of cutin	Enzymically liberated ml. equivalent 0·01 normal fatty acids
Gasteria verricuosa (Mill.) Duval	Leaf epidermis	$3{\cdot}4 \times 10^{-3}$
Solanum lycopersicum L.	Fruit epidermis	$8{\cdot}2 \times 10^{-3}$
Petunia hybrida (Hook.) Vilm.	Pistil cuticula	$3{\cdot}5 \times 10^{-3}$
Brassica nigra (L.) Koch	Stigma cuticula	$5{\cdot}2 \times 10^{-3}$

mustard stigma is attacked more than material from the pistil of *Petunia* and much more than cutin from leaves and fruits. The breakdown is measured by titration of the enzymically liberated fatty acids following a new method[5]. The differences in enzymic decomposition of cutin should depend on a specific state or a special composition of the high-polymeric, reticular substrate. For the first time it has been possible to demonstrate the enzymic breakdown of stigma cutin.

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MICROBIOLOGY

Mycobacteriophages isolated from Persons with Tuberculous and Non-tuberculous Conditions

In a recent communication[1] certain effects of mycobacteriophages on acid-fast bacilli were described. They consisted of the lysis of phage-susceptible bacteria, and of biological changes in the phage-resistant elements of the bacterial population. The phage-resistant bacteria were lysogenic. They showed changes in morphology, in the pigmentation of their colonies, in their susceptibility to mycobacteriostatic drugs, in their antigenic composition and in their pathogenicity for laboratory animals.

These *in vitro* observations raised the question as to whether or not similar changes may occur *in vivo*. Any work along this line had to start by demonstrating the occurrence of mycobacteriophages in the human or animal body.

Bacteriophages active on acid-fast micro-organisms have been isolated so far only from soil, particularly fertilized soil[2–5]. Hnatko[2] attempted to isolate mycobacteriophages from specimens of sputum and of stool from tuberculous patients, but he could not detect any phage activity in his filtrates.

Fifty specimens of stool from hospital patients with pulmonary tuberculosis were examined for the presence of mycobacteriophages.

10-gm. amounts of the specimens were emulsified in 200 ml. of Proskauer and Beck synthetic medium modified by Youmans and Karlson[6]. To this suspension were added 1-ml. amounts of actively multiplying cultures from 12 test strains of mycobacteria. These test strains included pathogenic and saprophytic mycobacteria. They were chosen because they apparently were free of prophages, that is, they did not show spontaneous or ultra-violet irradiation-induced lysogeny. After 10 days incubation at 37° C., the suspensions were filtered on membrane filters and the filtrates were added to fresh culture media inoculated with the test strains. After five such passages, the filtrates were spot-tested to determine lytic activity on any of the test strains. The technique for this screening procedure was described by Froman *et al.*[3] and modified by Takeya and Yoshimura[6].

Table 1 shows the lytic activity of ten mycobacteriophages which were isolated from ten of the fifty specimens of stool that were included in this study.

It is interesting to note that only one mycobacteriophage, isolated repeatedly from specimens of stool from patient D.W., is active on pathogenic mycobacteria. While at the time the specimens were collected the sputum cultures of the other nine patients yielded typical *M. tuberculosis*, those of patient D.W. consisted of atypical chromogenic acid-fast bacteria.

Specimens of stool were then collected from 30 patients with pulmonary conditions of non-tuberculous ætiology. Table 2 shows the bacteriolytic spectrum of five mycobacteriophages isolated from this group.

Patient McL., from whom a mycobacteriophage with a large lytic spectrum, including the virulent human strain $H_{37}Rv$, was isolated, came to this hospital to undergo resection for bronchogenic carcinoma. No history of tuberculosis was given. Four phages were isolated from patients with a diagnosis of sarcoidosis. From eight other patients

Table 1. Lytic Activity (*L*) of Ten Mycobacteriophages isolated from Tuberculous Patients

Mycobacteria	Bacteriophages from patients									
	S.G.	R.A.	L.C.	S.M.	W.O.	T.M.	B.R.	D.W.	L.A.	G.B.
Virulent human:										
$H_{37}Rv$	—	—	—	—	—	—	—	*L*	—	—
7101 *F*	—	—	—	—	—	—	—	*L*	—	—
Attenuated bovine:										
B.C.G.	—	—	—	—	—	—	—	*L*	—	—
Anonymous:										
Photochromogenic	—	—	—	—	—	—	—	*L*	—	—
Scotochromogenic	—	—	—	*L*	—	—	*L*	—	—	—
'Battey'	—	—	—	—	—	—	—	—	—	—
Fast-growing	—	*L*	—	—	—	—	—	—	*L*	*L*
Saprophytic:										
Phlei	*L*	—	*L*	*L*	*L*	—	—	—	*L*	—
Smegmatis	—	*L*	—	—	*L*	*L*	*L*	*L*	—	*L*
Fribergensis	*L*	*L*	*L*	*L*	—	—	—	*L*	—	—
Rabinowitschi	—	—	—	*L*	—	—	—	*L*	—	—
Stercoides	*L*	*L*	*L*	*L*	—	*L*	*L*	*L*	—	*L*

with the same diagnosis no mycophages were isolated from stool specimens.

The presence of mycobacteriophages in the stool of certain patients could either be temporary and accidental or it could be determined and maintained by infection with mycobacteria. In an attempt to approach this question, the following experiment was undertaken:

Five groups of 20 mice each (Rockefeller strain) were inoculated intravenously with either of two strains of mycobacteria. Three groups of mice were inoculated at the same time and by the same route with filtrates of mycobacteriophages to which the bacteria were susceptible, as follows:

Group 1 with *Mycobacterium smegmatis*.
Group 2 with *Mycobacterium smegmatis* and *Bacteriophage smegmatis* (courtesy of Dr. E. Vandra, Budapest).
Group 3 with the virulent human strain $H_{37}Rv$.
Group 4 with $H_{37}Rv$ and *Bacteriophage D*29 (courtesy of Dr. S. Froman, Olive View, California).
Group 5 with $H_{37}Rv$ and *Bacteriophage* D.W.

The amount of bacilli inoculated was 0·5 mgm. per mouse. The phage filtrates, from which 0·2 ml. were inoculated, contained 10^8 lytic particles per ml.

Specimens of stool were collected from each group of mice and were examined for the presence of mycobacteriophages. *Mycobacteriophage smegmatis* (Group 2) was lost: no phage could be recovered from these animals 48 hr. or more after inoculation. Specimens of stool from Groups 4 and 5 contained phages up to nine weeks after infection. According to their plaque morphology and their activity on mycobacteria, these phages were identical with *Mycobacteriophages D*29 and D.W., respectively.

Table 2. Lytic Activity (*L*) of Five Mycobacteriophages isolated from Non-tuberculous Patients

Mycobacteria	Bacteriophages from patients				
	McL.	McC.	G.W.	R.C.	R.L.
Virulent human:					
$H_{37}Rv$	*L*	—	—	*L*	—
7101 *F*	*L*	—	—	*L*	—
Attenuated bovine:					
B.C.G.	*L*	—	—	—	—
Anonymous:					
Photochromogenic	*L*	—	—	*L*	—
Scotochromogenic	—	—	—	—	—
'Battey'	*L*	—	*L*	—	—
Fast growing	—	*L*	—	—	—
Saprophytic:					
phlei	—	—	—	—	*L*
smegmatis	—	—	—	—	*L*
fribergensis	*L*	—	*L*	*L*	*L*
rabinowitschi	*L*	—	—	*L*	—
stercoides	*L*	—	*L*	*L*	*L*

When autopsies were performed on these animals nine weeks after infection, it was established that Groups 1 and 2, which were inoculated with *M. smegmatis*, did not show any gross pathology, whereas the mice inoculated with $H_{37}Rv$ showed lesions in kidneys and/or lungs.

The results of these animal experiments suggest that under certain conditions mice may remain infected with mycobacteriophages for long periods of time. One of the conditions appears to be the presence of multiplying mycobacteria in the tuberculous lesions.

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Release from the Feedback Inhibition controlling the Biosynthesis of Isoleucine

Hayashi *et al.* have found that a large quantity of L-isoleucine was accumulated in the cultured medium of *B. subtilis* strain 14 when α-aminobutyric acid (designated as *AB*) was added to the medium containing glucose, peptone, urea and mineral salts[1]. The apparent conversion ratio of *AB* to accumulated isoleucine reached 70 per cent when 1 per cent of the former was added to the medium. In spite of the nature of *AB* as a possible precursor of isoleucine, incorporation of the added *AB*-1-^{14}C to produced isoleucine was observed only at the initial stage of the culture[2]. As the culture proceeded to the accumulation stage of isoleucine, the specific activity of isoleucine tended to be decreased, which means that

isoleucine thus accumulated was derived from glucose. On the other hand, it is well recognized in yeast, *Neurospora* and *E. coli* that threonine is at first deaminated to α-ketobutyric acid, which then converts to isoleucine through several biosynthetic steps[2-6]. Further, it was depicted that biosynthesis of isoleucine was controlled by a feedback mechanism within the cells of *E. coli*[7,8].

We have examined the presence and the nature of threonine deaminase in *B. subtilis* strain 14, since it is one of the important enzymes in the most probable pathway of isoleucine biosynthesis, and in addition it was ascertained by Hayashi *et al.* that hydroxyacetyl butyric acid was converted to isoleucine by this strain[1].

An appreciable activity of threonine deaminase is detectable as compared with *E. coli*, and, indeed, specific activities (defined in the table) reached 2·0 to 3·0 in this strain compared with 9·0 in *E. coli* strain *K*12. Cells grown in the medium containing *AB* (*AB* cell) have usually lower activity as compared with those grown in the medium without *AB* (normal cell). Specific activities of the enzyme obtained from *AB* and normal cells, which were collected from 48-hr. cultures, ranged from 0·01 to 0·28 and from 0·13 to 0·63, respectively. In spite of this fact, which suggests that *AB* might repress or inhibit this enzyme through a certain mechanism, the enzyme activity was enough stimulated in either normal cells or *AB* cells when *AB* was added to the system of enzyme assay. In contrast, the enzyme activity was completely inhibited by the addition of 10^{-3} *M* isoleucine, as Umbarger pointed out a similar inhibition in *E. coli*[9]. An inhibition curve of sigmoid type was obtained between the rate of inhibition and the concentration of added isoleucine. As shown in Table 1, when isoleucine and *AB* were added simultaneously at various concentrations, the recovery from the isoleucine inhibition was observed according to the given concentrations of *AB*, where the inhibition curves by isoleucine shifted towards its higher concentrations. Leucine and valine, on the other hand, did not indicate any significant effect on the enzyme activity itself, but gave the similar effect as did *AB* on the inhibition by isoleucine.

Table 1. α-Aminobutyric Acid, Leucine and Valine as Releaser of the Inhibition by Isoleucine of Threonine Deaminase

Concentration of α-amino-butyric acid (*M*)	0	10^{-3}	10^{-2}	10^{-1}
Concentration of isoleucine (*M*)				
0	100*	105	118	132
10^{-4}	30·1	37·0	57·9	82·1
5×10^{-4}	2·1	3·7	4·5	48·8
2×10^{-3}	0	0	0	4·2
Concentration of amino-acids (*M*)		0	Leucine 2×10^{-3}	Valine 2×10^{-3}
Concentration of isoleucine (*M*)				
0		100†	80·7	104
5×10^{-4}		57·0	72·8	77·6
10^{-4}		26·6	65·8	63·8

* Figures represent relative activities to specific activity of 2·4.
† Specific activity, 1·93.

The cells were grown for a period of 24 hr. at 30° C. on a reciprocal shaker, using a medium of following composition: glucose, 10 per cent; peptone, meat extracts, each 0·2 per cent; urea, 0·5 per cent; K_2HPO_4, 0·07 per cent; $MgSO_4.7H_2O$, 0·03 per cent; $FeCl_3.6H_2O$, 0·005 per cent. In the case of *E. coli*, 1 per cent $CaCO_3$ was added to the medium.

The cells were collected by centrifugation, washed twice with 0·1 *M* phosphate buffer (*p*H 7·5) and suspended in the same buffer.

Deaminase activity was assayed at 37° C. in a 1-ml. system containing 40 μmoles of DL-threonine, 100 μgm. of sodium deoxycholate, 0·03 ml. of toluene, 0·4 ml. of cell suspension which contained 2–5 mgm. of dry cells and 40 μmoles of phosphate buffer (*p*H 7·5), and appropriate volume of inhibitor and releaser. After 1 hr. incubation, the reaction was stopped by the addition of perchloric acid. The extent of deamination was followed by determining the keto-acid formed using the modified method of Friedemann and Haugen (ref. 10). Specific activities are expressed in μmoles of keto-acid formed per hr. per 1 mgm. of dry cell.

From the results obtained here, it is postulated that the negative feedback mechanism in the biosynthetic process of isoleucine may exist also in *B. subtilis* strain 14, and the interference with this feedback mechanism may occur in the presence of *AB*, leucine and valine, but only *AB* and not leucine or valine leads to isoleucine accumulation. Moyed and Friedman indicated that feedback mechanisms controlling the biosynthesis of tryptophan and histidine had been lost in such mutant strains of *E. coli* resistant to their analogues[11]. Further, Adelberg indicated that mutant strains of *E. coli* resistant to thienylalanine or *p*-fluorophenylalanine, an analogue of phenylalanine or tyrosine, excrete a large amount of phenylalanine or tyrosine[12,13]. Another example of metabolite excretion was indicated by us, in which a type of releaser from the feedback inhibition may cause the excretion of a metabolite, which is usually supplied moderately by the feedback mechanism for further synthesis. *AB* is not an analogue of isoleucine, nor does not lead a resistant mutant in the above-mentioned medium (unpublished work).

As to the control mechanism, such as the negative feedback loop which may control the rate of biosynthesis of metabolite, it might not be allowed to act in such a simple manner *in vivo* as conceived from the results obtained *in vitro*, since many agents interfering with this mechanism would have to exist within the cell as indicated above.

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Spore Dispersal in *Chaetomium globosum* (Kunze)

In the genus *Chaetomium* the fruit-body, a perithecium, is ornamented with a large number of apical hairs which are either spirally coiled or dichotomously branched. The ascospores are released within the perithecium by breakdown of the ascal walls and the spores ooze out of the ostiole and either form a spore tendril or become entrapped in the apical hairs. Hitherto the mechanism of spore dispersal has been obscure, but recent experiments have shown that entire fruit-bodies as well as spores can be dispersed by falling water drops.

Target disks, 2 cm. in diameter, were cut from a three-week-old agar culture of *C. globosum* having approximately 150 perithecia per sq. cm. Disks were placed on glass slides and surrounded by small glass sheets. A single drop of water 5 mm. in diameter was allowed to fall from varying heights, up to 2 m., on to the centre of each target. This drop, on breaking up, cleared most of the perithecia from an area 0·5–1 cm. in diameter. The dispersed fruit-bodies were found scattered on the glass sheets either singly or in groups of up to twenty. The longest distance of dispersal recorded was 17 cm. The majority of perithecia were found in splash droplets, but others were dry and had obviously remained dry during dispersal. Other drops were found to contain ascospores and the maximum distance recorded for spore transport was 33 cm.

The ease with which perithecia can be dispersed by water is related to the very superficial position and loose anchorage of the fruit-body on the mycelium. The anchorage though loose is, however, resistant to wind. Experiments using a small wind-tunnel showed that with wind speeds up to 10 m./sec. the perithecia remained attached; the spores, however, were readily blown away from the spore masses trapped in the apical hairs.

The dispersal of perithecia in splash droplets and as dry fruit-bodies is probably related to the water-repellent nature of the fruit-body. Colonies of *Chaetomium* can be gently flooded with water without wetting the perithecia, the apical hairs retaining a bubble of air. The falling drop, however, has sufficient kinetic energy to break through the terminal hairs and to wet the perithecia at the point of impact. The unwetted perithecia are probably picked up as the impact drop breaks up into the splash droplets. These small drops would strike the perithecia laterally, the latter being carried on the surface of the drop from which they may be shed during the free flight.

This method of splash dispersal involving whole fruit-bodies appears to be unique in the fungi. Falling water drops act as the agent of dispersal in many bacteria and slime-spored fungi[1] and work by Gregory *et al.*[2] has established the basic mechanics involved. Comparable examples of the splash dispersal of larger units are few and are confined to the dispersal of the lenticular peridioles in the Nidulariales and the sclerotial balls of *Aleurodiscus minnsiae*[3].

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GENETICS

Fracture and Fusion of Coccid Chromosomes

Unlike gene mutations the expression of which may be subject to considerable modification, chromosomal changes will remain the same whether they occur as sporadic aberrations or become incorporated in the karyotype of a species. If the complements of two closely related species differ, spontaneous chromosomal mutations paralleling the karyotypic differences should be demonstrable in the same group of organisms.

In the Coccoidea, the haploid chromosome number ranges from 2 to 20 (ref. 1). Since the basic number for the superfamily is in the range of $n = 5$ to 7, both increase and decrease have occurred in the evolution of the karyotype. The chromosomes of the coccids lack localized centromeres and fragments perpetuate themselves during successive mitotic cycles[2]. Thus, simple breakage can provide the basis for increase in chromosome number unless a breakage-fusion-bridge cycle intervenes to eliminate the breakage products. Fragmentation with and without subsequent bridge formation has been previously reported[2]. When bridges are formed, either spontaneously[3] or after high-dosage irradiation[4], they are unmistakably identifiable in my preparations. Their absence in other circumstances indicates that fracture *per se* need not lead to a breakage-fusion-bridge cycle.

Related coccid species may vary in number by one or two chromosomes or by exact doubling. The latter changes, similar to polyploidy, are complex problems and will not be considered here, but parallels of structural aberration and karyotype change are now evident for the simple increases and decreases.

Spontaneous chromosome breakage occurs ubiquitously in the coccids[3–5]; there is no doubt of the abundant availability of ruptured chromosomes for increase in number should chance or selection so dictate. Three cases are now known in which karyotypes of related species differ in the manner expected from fragmentation. On the basis of relative measurements, the karyotype of *Africaspis fici* with $n = 6$ chromosomes appears derivable by simple fragmentation from that of *A. chionaspisformis* with $n = 4$ (ref. 5). In this instance, the chromosomes of *A. fici* varied considerably more in size than those of its congener; however, size variation can be achieved through unequal translocation while breakage of the longest chromosome of a complement could lead to more nearly uniform sizes in the derived karyotype. A Mexican species with $n = 6$ is now known from the *Aspidiotus-Hemiberlesia* series and is the first departure, other than that of a highly atypical genus[5], from the basic diaspidid number, $n = 4$, among the numerous species of Aspidiotini examined (Fig. 1*A*). Among the endemic Hawaiian pseudococcids, the genus *Pedronia* is noteworthy for its association with ferns[6]. In two of the species studied cytologically, the basic number of the pseudococcids, $n = 5$, was found while in a third the number was 7 (Fig. 1*B*). In the last two examples, the increase of the haploid number by two required, on a minimal assumption, the rupture of two chromosomes to yield four breakage products. On the basis of size criterion, no breakage products can be identified in the Mexican species and only one or two in the *Pedronia* example; in both cases, therefore, breakage would have had to occur in the longer chromosomes of the antecedent karyotypes. Forms related to the Mexican species are as yet unknown, but the complements of the two *Pedronia* species with $n = 5$ show the expected size variation.

Fusion of the major portions of two chromosomes by means of a reciprocal translocation at sub-terminal points of breakage and loss of the small residual chromosome will reduce the chromosome number by one. The 'double-length' chromosome will be detectable in the karyotype unless its size is reduced afterwards by additional translocations or the original fusion involves two especially short chromo-

Fig. 1. Structural alterations of coccid chromosomes. *A*, Aspidiotine species, *Aspidiotus-Hemiberlesia* complex, $n = 6$. *B*, *Pedronia cibotii*, $n = 7$; arrows, possible fragmentation products. *C*, Aberrant strain of parthenogenetic sp., *Howardia biclavis*, $2n = 10$; arrows, 'double-length' and residual chromosomes from unequal translocation. *D*, *Pseudococcus adonidum*, $n = 4$; arrow, 'double-length' chromosome

somes. A spontaneous change of this sort has been reported for an ovarian mosaic of an armoured scale insect[2]. A somewhat similar alteration, with a residual product undoubtedly too large for tolerable loss, has now been found in a collection of the parthenogenetic armoured scale, *Howardia biclavis* with $2n = 10$ (Fig. 1*C*); because ōogenesis is here essentially mitotic, the occurrence in the sample of several individuals with the aberration is a reflection of the clonal type of reproduction. In the *Pseudoparlatoria* complex in North America, species have been recorded with $n = 4$, and one member of double length, and with $n = 5$ (ref. 5). There is no definitive evidence to show the direction in which the change occurred; although 4 is the basic diaspidid number, the 4-chromosome complement in question is highly distinctive and was most likely secondarily derived. A much simpler example is provided by *Pseudococcus adonidum* with $n = 4$; this, the only reduction yet known from the basic number, $n = 5$, of the pseudococcids, is accompanied by a double-length chromosome (Fig. 1*D*). In the armoured scale genera, *Protodiaspis* and *Ancepaspis*, species with $n = 4$ and $n = 3$ have been discovered, and in both cases a disproportionately long chromosome occurs in the species with the lower number. These two genera present an interesting phylogenetic problem.

The method of accounting for karyotypic changes on the basis of the evidence from spontaneous alterations in the same group seems to afford a better understanding of the evolutionary process than inferences from work with dissimilar organisms, no matter how conclusive they seem in themselves.

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Discriminant Analysis in Biometrical Genetics

Several notable attempts have been made to analyse and interpret the inheritance of polygenic characters in terms of simple Mendelian factors giving the familiar ratios in segregating generations[1,2]. More recently, Weber[3,4] proposed his so-named discriminant analysis technique, in which two or more component measurements of a character that may individually appear to be normally distributed are combined into a discriminant function which, in contrast, might assume generally a bimodal frequency distribution suggesting this linear compound measure to be simply inherited like qualitative traits. He illustrates this method for the cotyledon size in tomato by using a linear function of length/width ratio (x_1) and area (x_2), $x = b_1x_1 + b_2x_2$, and showing x to have a bimodal frequency distribution in F_2 that simulated a segregation ratio of 3:1 in one case and of 9:7 in the other. Apparently, Dr. Weber considers this method of analysing quantitative characters to be useful in general as he argues[3], "this total measurement of discriminant function has no dimensional designation; it is without dimension in the physical sense, like the term red or white colour".

While this new analysis in biometrical genetics may have some appeal, for its empirical value in particular, to those used to thinking only in terms of simple gene models and Mendelian ratios, it is, however, open to criticism on such grounds as the applicability of the method in general and its genetic implications. In statistical theory, it is well known that a linear function of several normal variables is also distributed normally, so that a bimodal distribution of the discriminant function such as that observed by Weber for the cotyledon size in tomato would seem to be very unlikely unless the sampling errors were rather large or the component characters were actually non-normal variates. In any event, this method might be applicable to some very rare examples merely for expediency rather than as a real solution. Moreover, it seems quite inexplicable how several polygenic systems governing the inheritance of various individual components could be made to behave like one or two genetic units by merely considering their segregation together. This, in fact, appears contrary to the usual approach of breaking down a complex character such as yield into its components that may involve relatively simpler genetic situations[5]. Here, Dr. Weber perhaps argues that it is the total impression of a character which is under genetic control while the dimensional measures are artefacts from the viewpoint of genetic determination. Obviously, such an argument would seem to imply the non-existence of a polygenic mode of inheritance of many of the quantitative characters. Thus, it appears that a critical evaluation of this method of discriminant analysis is quite in order in view of an important issue on the polygenic inheritance being involved, and that many more examples need to be investigated before even its empirical usefulness is well demonstrated.

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FORTHCOMING EVENTS

(Meeting marked with an asterisk (*) is open to the public)

Tuesday, October 3

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH-EASTERN BRANCH at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. Calderbank: "The Origins and Purpose of Chemical Engineering" (Inaugural Lecture).

Tuesday, October 3—Thursday, October 12

ELECTRONIC COMPUTER EXHIBITION (at Olympia, London).*

Wednesday, October 4

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5 p.m.—Mr. A. R. Thatcher: "Some Results on Inventory Problems".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. H. G. Priest: "The Packaging of Lubricants".

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. D. A. Drew: "Methods Used for the Study of Vibration in Aero Engines".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. F. T. Barwell: "Some Speculations on the Future of Railway Mechanical Engineering".

Thursday, October 5

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. S. C. Lucas: Inaugural Address as President.

Thursday, October 5—Friday, October 6

BIOCHEMICAL SOCIETY (joint meeting with the BRITISH BIOPHYSICAL SOCIETY, in the School of Pharmacy, University of London, Brunswick Square, London, W.C.1).

Thursday, October 5

At 11 a.m.: "The Physical Chemistry of Phospholipids".

Friday, October 6

At 10.30 a.m.: "Phospholipids and Sulpholipids".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Institution of Mechanical Engineers, Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m. on Thursday and 10.30 a.m. on Friday—Meeting on "Advances in Analytical Chemistry—Selected Topics Originating from Analytical Requirements in the Field of Nuclear Technology".

Friday, October 6

PHOTOELECTRIC SPECTROMETRY GROUP (in the Department of Physics, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 2.30 p.m.—Annual General Meeting. 3.15 p.m.—Meeting on "Absorption Measurements at Wave-lengths Below 200 mμ".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (in the Wellcome Building, Euston Road, London, N.W.1), at 2.45 p.m.—Meeting on "Approaches to the Chemotherapy of Virus Diseases".

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "What is *p*H and Its Measurements?" opened by Dr. S. Mattock and Mr. G. R. Taylor.

Saturday, October 7

BIOCHEMICAL SOCIETY (at the Westminster Hospital Medical School, Horseferry Road, London, S.W.1), at 10 a.m.—Ordinary Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with a good honours degree in electrical engineering and preferably with special interests in electrical machinery) IN ELECTRICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 6).

DEMONSTRATOR (with medical qualifications registrable in this country) IN ANATOMY—The Registrar, The University, Manchester 13, (October 7).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER, to assume responsibility for the supervision and maintenance of electronic control, computer and digital equipment associated with the radio telescope and other apparatus at Jodrell Bank—The Registrar, The University, Manchester 13 (October 7).

LECTURER AND ADMINISTRATIVE OFFICER (with a university degree in science and with good qualifications in physics, preferably with a knowledge of Welsh) IN THE FACULTY OF EDUCATION—The Registrar, University College, Cathays Park, Cardiff (October 7).

ASSISTANT CHIEF OFFICER (with a degree in botany, agricultural botany or agriculture or equivalent qualifications, and preferably experience of seed improvement and staff administration) AT THE OFFICIAL SEED TESTING STATION—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (October 9).

ASSISTANT LECTURER or LECTURER IN GENETICS—The Registrar (The University, Sheffield (October 14).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (October 14).

RESEARCH FELLOWS (2) (with an honours degree and three years postgraduate experience in a relevant field) IN SEISMOLOGY IN THE DEPARTMENT OF ASTRONOMY, for the investigation of local structure and local earthquakes, and the interpretation of dispersed seismic waves—The Secretary, The University, Edinburgh (October 14).

LECTURERS or ASSISTANT LECTURERS (with a good honours degree or equivalent qualification, preferably with teaching and/or research experience) IN GEOLOGY at the University, Kuala Lumpur, Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

ASSISTANT LECTURER (with a good honours degree in zoology and preferably a special interest in the arthropods) IN INVERTEBRATE ZOOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 16).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER IN THE DEPARTMENT OF METALLURGY, to operate, maintain and develop an electron beam micro-probe analyser—The Registrar, The University, Manchester 13 (October 16).

SENIOR LECTURER and a LECTURER IN MECHANICAL ENGINEERING at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 23).

LECTURER or SENIOR LECTURER IN PURE MATHEMATICS at the University of New England, Armidale, N.S.W., Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 27).

CURATOR (with a qualification in zoology and preferably experience of a teaching museum) OF THE ZOOLOGY MUSEUM—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

LECTURER/SENIOR LECTURER (with a particular interest and qualifications in the philosophical and/or sociological aspects of education) IN EDUCATION at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

LECTURER IN PURE MATHEMATICS at the R.A.A.R. Academy, which is affiliated with the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

LECTURER (with an honours degree in mining engineering or equivalent, and considerable experience in metalliferous mining) IN METALLIFEROUS MINING—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (October 31).

NUFFIELD RESEARCH FELLOW (preferably with an interest and experience in theoretical or experimental neutron physics)—IN THE DEPARTMENT OF PHYSICS—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (October 31).

READER (with teaching and research experience and with published work in the field of economic statistics, preferably with a knowledge of econometries) IN ECONOMIC STATISTICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 31).

FOOD TECHNOLOGIST (preferably with a Ph.D. in food technology or postgraduate experience in this field) IN THE FRUIT RESEARCH DIVISION, D.S.I.R., Stoke, near Nelson, New Zealand, for the investigation of problems arising in the processing of fruits and vegetables, and the development of new products from these crops—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B.13/18/2 (November 16).

LECTURER IN PHILOSOPHY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 18).

LECTURER IN EDUCATION at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 20).

PLANT PHYSIOLOGIST (with postgraduate experience), to undertake studies on the growth of the tobacco plant—The Director, Tobacco Research Board of Rhodesia and Nyasaland, P.O. Box 1909, Salisbury, Southern Rhodesia (November 30).

ANALYST (B.Sc.(chemistry) and preferably G.R.I.C. or equivalent qualifications), for the operation and care of optical equipment including infra-red and nuclear magnetic resonance spectrometers—Mr. W. A. Wightman, School of Chemistry, The University, Leeds 2.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree, preferably in zoology and chemistry) IN THE UNIT FOR BIOCHEMICAL RESEARCH ON FISHERIES' PROBLEMS—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Berks, quoting Ref. 61/24.

ASSISTANT LECTURER IN CHEMISTRY—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

ASSISTANT LECTURER (with a good honours degree and preferably with research and/or industrial experience) IN PHYSICAL CHEMISTRY—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

EDITORIAL ASSISTANT (with a degree in science and preferably editorial experience) for the *Biochemical Journal*—The Editorial Board, The *Biochemical Journal*, 133–135 Oxford Street, London, W.1.

LECTURER (B.Sc. (chemistry and biology) and preferably with a teaching diploma), in Nigeria to teach science subjects to pharmacy students—The Recruitment Secretary (Overseas), Northern Region Recruitment Agency, Nigeria House, 9 Northumberland Avenue, London, W.C.2.

LECTURER (graduate of a British university) IN PHYSIOLOGY IN THE DEPARTMENT OF HEALTH EDUCATION—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

PHYSICIST (with an honours degree and preferably with appropriate research experience), to take part in a programme or work concerning the physical properties of milk—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/27.

RESEARCH ASSISTANT (preferably with research experience) IN THE DEPARTMENT OF GENETICS, to work in collaboration with Prof. J. A. Roper on nucleo-cytoplasmic interactions in fungi—The Registrar, The University, Sheffield.

RESEARCH WORKER (with a qualification in chemistry and preferably previous experience in polymer chemistry) IN THE ISOTOPE UNIT, for work in the field of polymer reaction mechanisms—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

SENIOR TECHNICIAN or TECHNICIAN (with experience and training in electronics) IN THE PHARMACOLOGY DEPARTMENT—The Secretary, Royal Free Hospital Medical School, 8 Hunter Street, London, W.C.1.

REPORTS and other PUBLICATIONS

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Great Britain and Ireland

British Association for the Advancement of Science. Annual Report, 1960–61. Pp. 31. (London : British Association for the Advancement of Science, 1961.) [148

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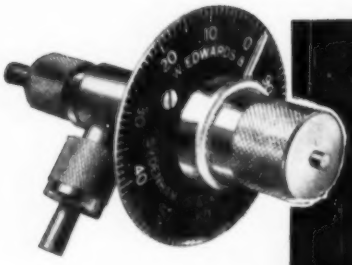

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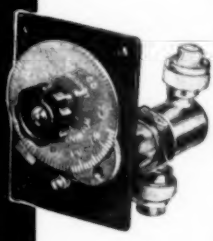

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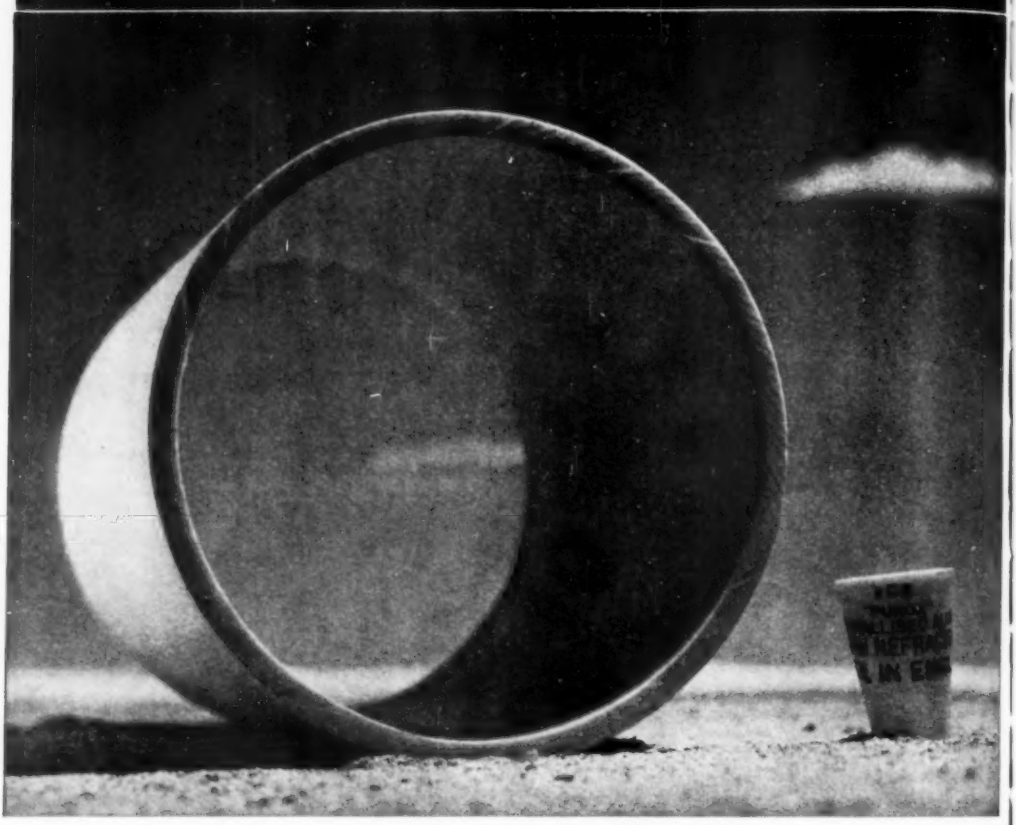

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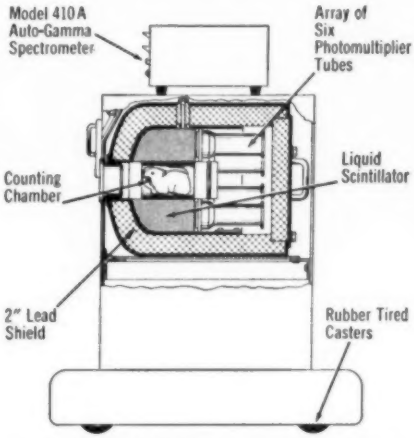

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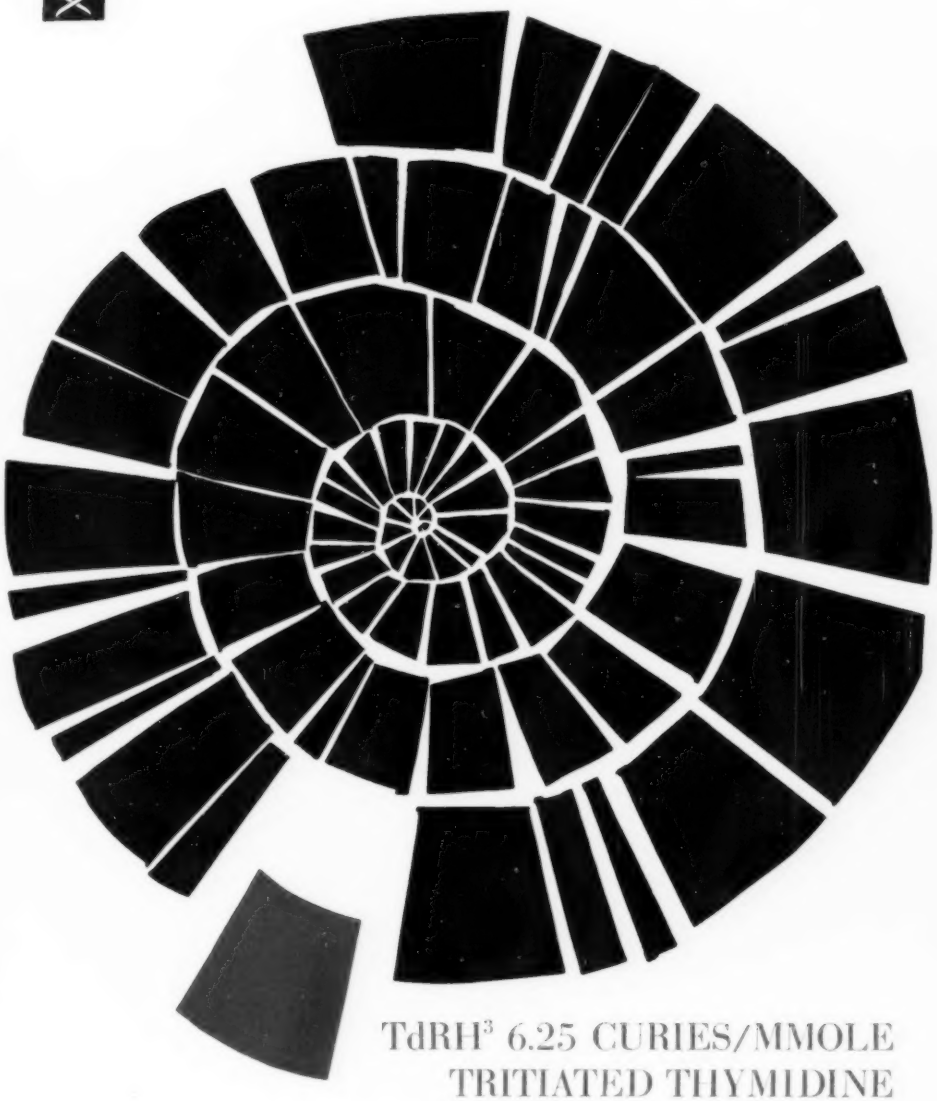

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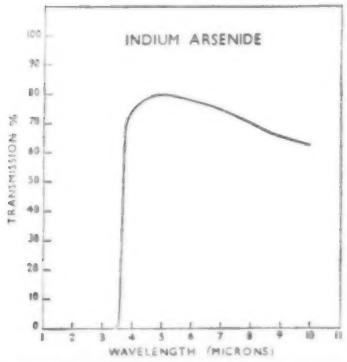

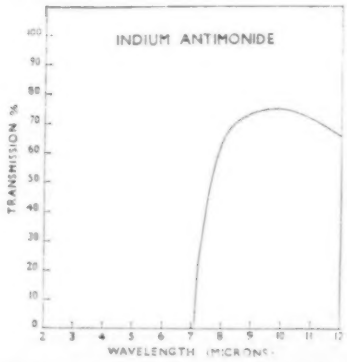

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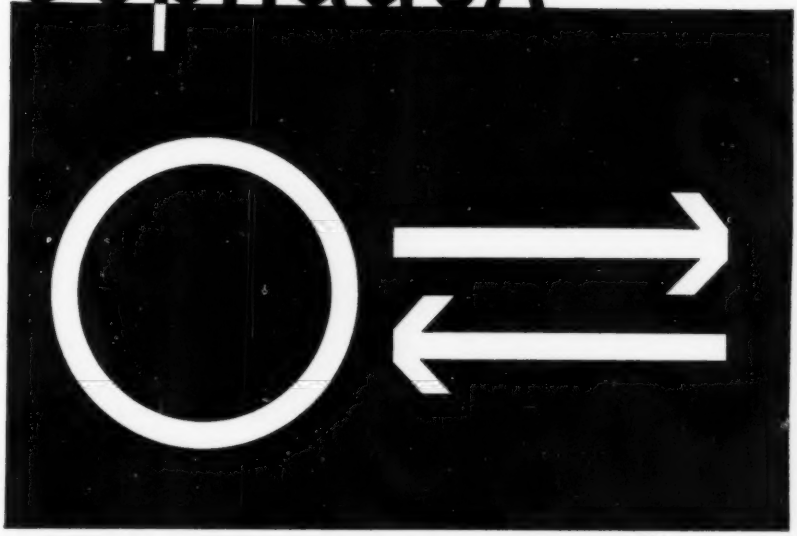

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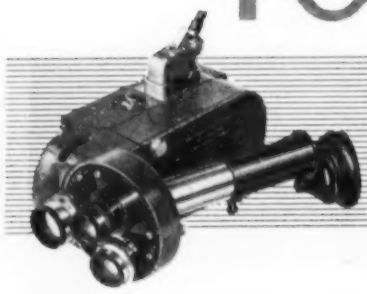

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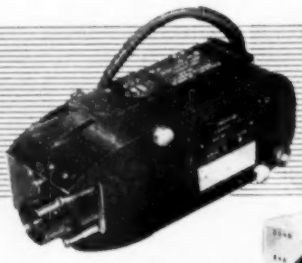

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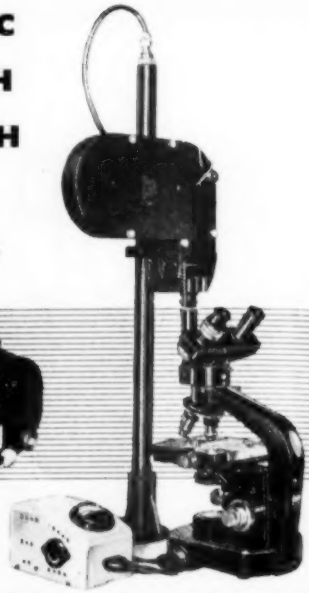

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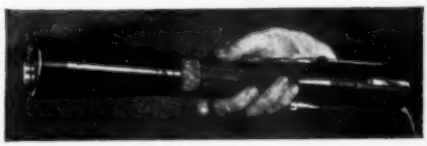

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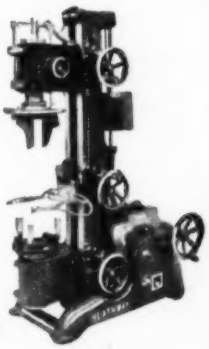

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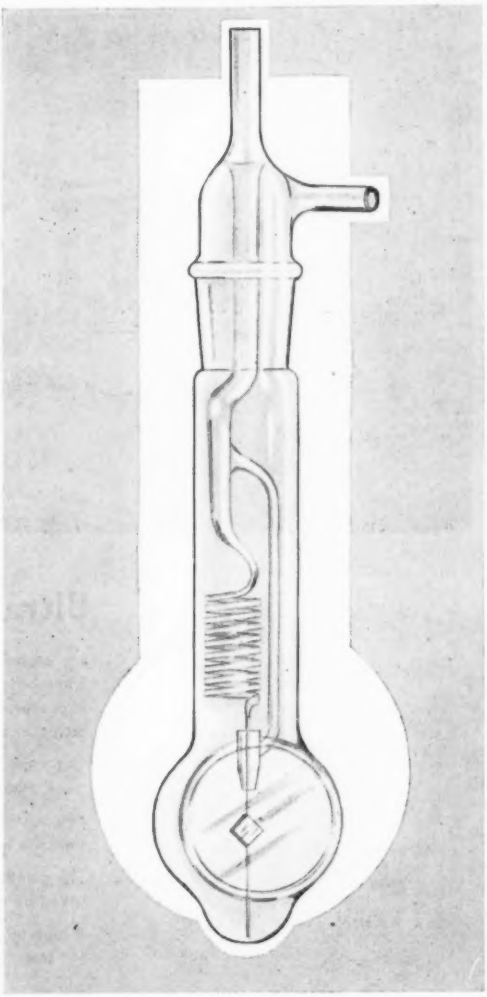

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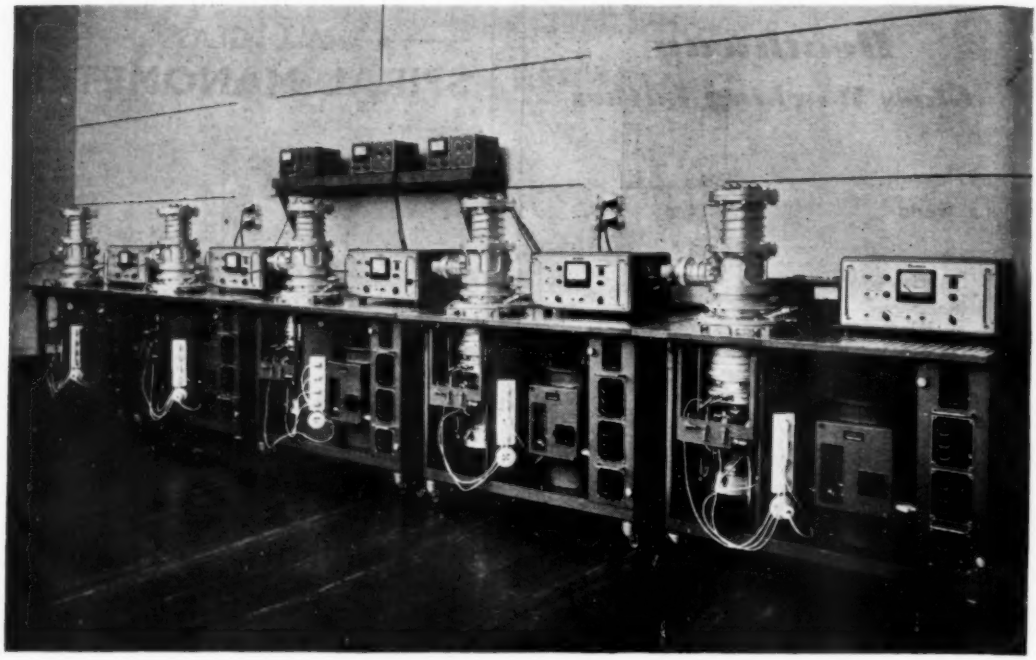

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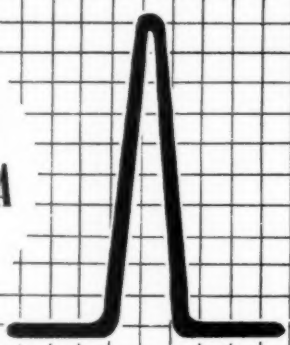

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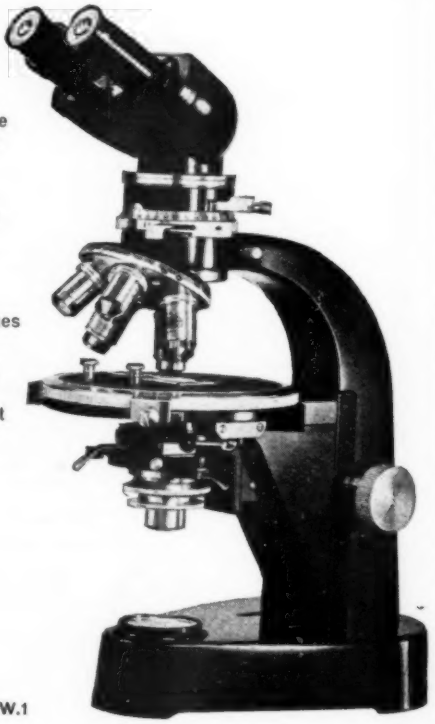

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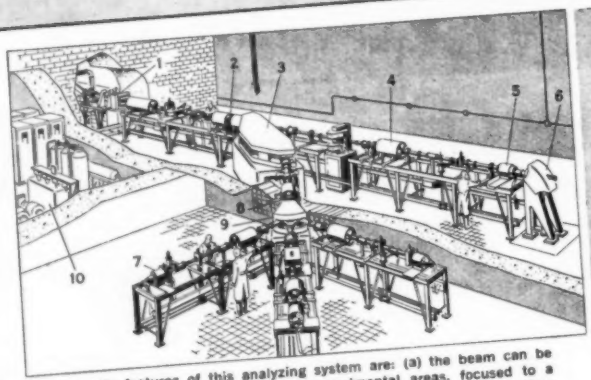

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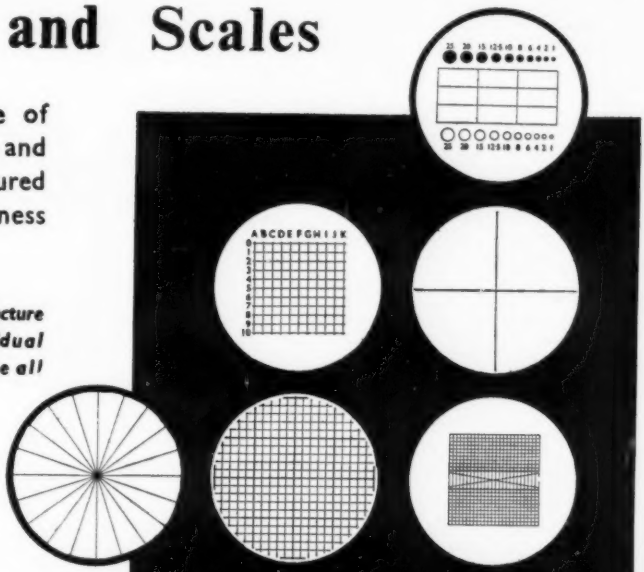

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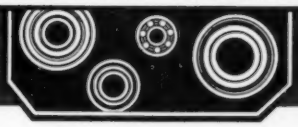

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BARKING, ESSEX

CHEMICAL ENGINEERING

A Chemical Engineer is required to be concerned with the development of processes for practical introduction into the factories. The post is likely to be of interest to a person of about 24-30, preferably holding a postgraduate qualification in chemical engineering but those holding a good degree in chemistry or chemical engineering and wishing to qualify for A.M.I.Chem.E. are also invited to reply.

Ref. M.165

FILTRATION MATERIALS FOR LIQUIDS AND GASES

This is a new post which will be concerned with the study of filtration problems and will involve supervision of the development and testing of new materials. It calls for a qualified scientist preferably with experience of filtration materials. **Ref. M.166**

Responsibility for both posts is to the **Senior Chemical Engineer.** *They are considered to be important appointments and therefore carry salaries which will attract the right men. There are good prospects for technicians within the Group and general conditions of service, including Pension and Life cover, are excellent.*

Applications, which will be accepted in complete confidence, should include full personal details, including age, qualifications, experience and present or required salary, and should be addressed quoting the relevant reference number to :

The Group Personnel Manager, The Cape Asbestos Co. Ltd., 114-116 Park Street, London, W.1.

Appointment of Director of Research

Benger Laboratories Limited, a member of the Fisons Group, has an important vacancy for a Director of Research, following the resignation of Dr. L. Golberg, who has been appointed Director of the British Industrial and Biological Research Station.

The new appointment is for a chemist of high attainments and considerable experience within the pharmaceutical field. His task will be to lead a well established team including chemists, pharmacologists, biochemists, bacteriologists and their ancillary staff, in the search for physiologically active compounds capable of being developed as ethical pharmaceuticals.

The Research Department is well housed and well equipped, and is located at the Company's manufacturing establishment at Holmes Chapel, Cheshire. At the present time the Research Department includes twenty graduate scientists and fifty ancillary staff. A progressive view is taken on the importance of such research in the Company's programmes and for its progress.

The Director of Research is expected to work in close co-operation with the Technical Director and the Chief Medical Adviser who are respectively responsible for product development and clinical assessment.

The research problems involved in this appointment are full of challenging interest and offer great scope for exploratory studies into structure-activity relationships. The Director of Research must possess the attributes of enthusiasm, leadership, co-operation with others, and an urgent desire to make real contributions to knowledge of chemical pharmacology and to the development of new ethical pharmaceuticals. The appointment will be well remunerated and excellent contributory pension schemes are in operation.

Applications, which will be treated in strict confidence, should be sent to the Secretary, Benger Laboratories Limited, Holmes Chapel, Cheshire.

TOBACCO RESEARCH BOARD OF RHODESIA AND NYASALAND

Applications are invited for the appointment to the Board's staff of a

PLANT PHYSIOLOGIST

to undertake studies on the growth of the tobacco plant. Postgraduate experience is essential. Salary grade: £1,500 to £2,400. The commencing salary will be determined by qualifications and experience.

Travelling and reasonable incidental expenses to Salisbury for the successful applicant and any family will be refunded by the Board. Conditions of service include 30 days annual leave, accumulative to a maximum of four months, plus occasional leave at the rate of 12 days per annum. There is also a Provident Fund and Medical Aid Scheme, membership of which is obligatory.

Applications, giving full personal details and experience, as well as the names of two referees, should be addressed under confidential cover to reach the Director, P.O. Box 1909, Salisbury, S. Rhodesia, not later than November 30, 1961.

UNIVERSITY OF OTTAWA
OTTAWA, ONTARIO, CANADA

Assistant Professor in Pharmacology required, Ph.D. or M.B. Salary $6,000 to $8,500 according to qualifications and experience.

Applications to Professor M. F. Murnaghan at the above address.

XUM

COMPUTER METHODS
and
POPULATION GENETICS

To carry out studies in human population genetics using computer methods. The position offers an opportunity to participate at an early stage in the development of an essentially new kind of population study using one of the newest and fastest computers in Canada.

Applicants (male or female) with university training and experience in statistics, computer programming, or population genetics preferred.

Salary will depend on qualifications.

Please include all details in first letter to:

File 9 C.
The Employment Supervisor,
ATOMIC ENERGY OF CANADA LIMITED,
Chalk River, Ontario.

WOOL RESEARCH ORGANIZATION OF NEW ZEALAND (INC.)
CHEMIST

The Wool Research Organization of N.Z. (Inc.) wishes to engage a well qualified chemist, with an interest in microbiology and enzyme chemistry, for research relating to the production of slipe wool.

This is an opportunity to join a large new research organization and to study wool, particularly in relation to skin structure and composition, depilatory substances and enzyme de-woolling.

Initially, these investigations will be carried out in the laboratories of another research institution at Wellington. In about four years' time the appointee will be transferred to the Organization's laboratories which are to be built at Lincoln, near Christchurch.

Salary will be, according to qualifications and experience, up to £2,300 a year. Superannuation is available. Fares to New Zealand and other expenses provided.

Applications, stating details of age, qualifications and experience, should be addressed to:
The Secretary,
Wool Research Organization of N.Z. (Inc.),
P.O. Box 5115,
Wellington, New Zealand.

Further particulars are available from the above or from the New Zealand Scientific Liaison Officer, Africa House, Kingsway, London, W.C.2.
Applications close October 31, 1961.

DEPARTMENT OF SCIENTIFIC AND INdustrial Research. Two pensionable posts at National Engineering Laboratory, East Kilbride, Glasgow, for men or women as (a) Principal Scientific Officer and (b) Senior Scientific Officer to conduct research into the application of servo control to machine tools. Applicants for post (b) must be under 40 on August 1, 1961. Qualifications: normally first- or second-class honours degree in engineering, or high professional attainment; at least three years' postgraduate or other approved experience; experience of electrical servo design and development; good knowledge of servo theory. A knowledge of hydraulic servo technique or machine tool design is an advantage. National salary scales (under review): (a) £1,716 to £2,418; (b) £1,342 to £1,654. Starting salary may be above minimum. Promotion prospects. Houses available for married staff.—Write Civil Service Commission, 17 North Audley Street, London, W.1, for application form, quoting S/5390/61. Closing date October 26, 1961.

CHEMISTS FOR DEFENCE RESEARCH BOARD, CANADA

SUFFIELD EXPERIMENTAL STATION, RALSTON, ALBERTA

ORGANIC CHEMIST.—Studies of chemistry of biologically active compounds. Special interest in chemistry of nitrogen and phosphorous in organic compounds.

PHYSICAL CHEMIST.—Original research into the relationship between the physical, chemical, and structural properties of biologically active compounds, and the effects of these compounds.

The Suffield Experimental Station conducts programmes in physical, chemical, biochemical, physiological, and bacteriological research, and offers modern, low-cost housing and excellent educational and recreational facilities in the Crown Village of Ralston.

NAVAL RESEARCH ESTABLISHMENT, HALIFAX, NOVA SCOTIA

PHYSICAL-ORGANIC CHEMIST.—Investigation of materials to convert mechanical energy to electrical energy. Special study of properties of organic molecules showing piezo-electricity and structural features producing this effect leading to development of polymer type piezo-electric material suitable for transducers.

CANADIAN ARMAMENT RESEARCH AND DEVELOPMENT ESTABLISHMENT, VALCARTIER, QUEBEC

POLYMER CHEMIST.—Preparation of fluorine compounds and polymers, study of application of polymers to rocket propellants, and kinetic studies of compounds in relation to polymer potentiality. Objective to prepare elastomeric polymer with high N-F and S-F content.

PHYSICAL-ORGANIC CHEMIST.—Studies of mechanism by which propellants degrade with age, using such techniques as differential thermal analysis, infra-red, ultraviolet, and gas chromatography, and mass spectrometry.

Qualifications.—Advanced degree from a recognized university, Ph.D. preferred, with originality and sound research background.
Citizenship.—Must be Canadian citizen or British subject.
Salary.—Starting salaries up to $10,000 depending on qualifications and experience.
Facilities.—These openings offer research chemists interesting careers in modern, well-equipped laboratories, with opportunity for presentation of papers in open literature and at scientific meetings.

Please write, outlining your qualifications and experience, to:
Defence Research Member,
Canadian Joint Staff,
1 Grosvenor Square, London, W.1,
and refer to file 61-*DRB*-2.

M&B

RESEARCH IN WEED CONTROL

MAY AND BAKER LTD, Ongar, Essex, have a vacancy at their Agricultural/Horticultural Research Station, Ongar, Essex, for a Botanist or other plant scientist with a liking for work with practical objectives.

He or she will be required to carry out greenhouse investigations into the potentialities, both home and overseas, of new herbicides discovered in screening tests. A good honours degree and at least three years research experience are necessary, as well as the ability to take charge of a small research team; age under 35. Experience can be in some aspect of experimental plant biology not necessarily with weeds, but the successful candidate will be expected to learn and keep an interest in weed and weed-killing problems. There is a Pension Fund and Life Assurance Scheme in operation. Five-day week and canteen facilities.

Apply initially in writing, to the Personnel Officer, giving full particulars of qualifications and experience.

UNIVERSITY OF LEEDS
PROCTOR DEPARTMENT OF FOOD AND LEATHER SCIENCE

Applications are invited for appointment as Lecturer or Assistant Lecturer in Food Science at a salary on the scale £1,050 by £50 to £1,400 by £75 to £1,850 a year for a Lecturer (efficiency bar at £1,550) and within the range £800 to £950 a year for an Assistant Lecturer, according to age, qualifications and experience. This is a post created to assist in new courses for B.Sc. honours and B.Sc. ordinary degrees in Food Science, which commence October 1, 1962. The duties will include responsibility for a laboratory containing small scale plant. Applicants should have an honours degree and have had practical experience of at least one branch of the food industry.

Applications (three copies), stating date of birth, qualifications and experience, together with the names of three referees, should reach the Registrar, the University, Leeds, 2 (from whom further particulars may be obtained), not later than October 14, 1961.

UNIVERSITY OF MANCHESTER

Applications are invited for appointment as Experimental Officer or Senior Experimental Officer in the Department of Metallurgy. The successful candidate will be required to operate, maintain and develop an electron beam micro-probe analyser in the Department of Metallurgy. His responsibilities will include necessary adjustments to the machine in conjunction with the suppliers, the preparation of specimens and some experimental work in conjunction with use of the machine in the investigation of the structure of metallic meteorites and work on diffusion. Salary range: £700 by £30 to £790 by £40 to £1,000 per annum (Experimental Officer): and £1,000 by £50 to £1,250 (merit bar line) by £50 to £1,500 (Senior Experimental Officer). The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 16, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

Wall's ICE CREAM

We have the following vacancies in our Laboratory at Acton, W.3.

PHYSICAL CHEMIST

(Ref. 356)

To carry out research on food products. Candidates should have an Honours degree in Physics or Physical Chemistry. Experience or interest in any of the following fields : colloids, emulsion, Rheology, will be an advantage.

ASSISTANT GRADUATE ANALYST

(Ref. 367)

The work involved is essentially routine analysis with some investigation of new analytical methods. Candidates should preferably be under thirty years of age.

* Starting salary will be commensurate with qualifications and experience, plus the usual social security amenities of a large and progressive Company.

* Applications in writing to the Company Personnel Manager, T. Wall & Sons (Ice Cream) Ltd., Wall's House, Olympic Way, Wembley, Middlesex, quoting the appropriate reference.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

CHEMIST, CHEMICAL ENGINEER OR PHYSICIST

The Organization's **Chemical Engineering Section, Melbourne, Victoria,** invites applications for a position of Chemist, Chemical Engineer, or Physicist. Applicants should possess a University Honours Degree or its equivalent in Chemical Engineering, Chemistry, or Physics together with some years of research experience.

The appointee will be required to undertake work of a fundamental nature in the fields of fluid dynamics, mass transfer, and reaction kinetics. In the early stages the work will be concerned mainly with mass transfer across liquid-liquid interfaces in reacting and non-reacting systems, but will be extended later to cover other types of contactor, and also chemical reactors.

Salary: Dependent on qualifications and experience within the ranges: £A.1,541 to £A.2,296 per annum or £A.2,426 to £A.2,751 per annum.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

By mutual arrangement the appointment may be either permanent or for a fixed term of three years.

Fares paid, and for a fixed-term contract return fares also paid. Further particulars supplied on application to :

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2.

to whom applications (quoting Appointment No. 608/15) should be addressed by October 28, 1961.

UNIVERSITY OF LONDON
KING'S COLLEGE

The College will require on January 1, 1962, an Assistant Lecturer in Invertebrate Zoology. Applicants should have a good honours degree in zoology. Preference will be given to an applicant with special interest in the arthropods. Salary scale: £800 by £50 per annum to £950, plus a London allowance of £60, family allowances and F.S.S.U. benefits.

Full conditions of appointment and application forms may be obtained from the Registrar, King's College London, Strand, W.C.2, completed forms must reach him by October 16.

WESTERN AUSTRALIA
STATE PUBLIC SERVICE
ASSISTANT ASTRONOMER
GOVERNMENT OBSERVATORY, PERTH
(Permanent)

Salary Range:	£A.1,515 to £A.2,163 (gross) per annum on current basic wage according to qualifications and experience.
Duties and Qualifications:	Main duties relate to research work in positional astronomy with particular emphasis on meridian astronomy. Applications should contain full information of academic and other qualifications and experience in the meridian astronomy field. References should be submitted. If any further details of duties required they may be obtained from Mr. H. S. Spigl, Government Astronomer, Observatory, Perth, Western Australia.
Transport:	Reasonable amount allowed for transport of appointee and dependent family to Western Australia provided bond entered into to serve State for period of three years.
Conditions of Service:	Superannuation benefits, three months' long service leave after seven years' continuous service, cumulative sick leave entitlements and other general conditions applicable to permanent public servants.

Applications, stating age, qualifications and experience, should be addressed by December 31, 1961, to:

Public Service Commissioner,
184 St. George's Terrace,
PERTH,
WESTERN AUSTRALIA.

FOURAH BAY COLLEGE
THE UNIVERSITY COLLEGE OF SIERRA LEONE

Applications are invited for appointment as Laboratory Superintendent in the Department of Engineering. Salary scale: £660 by £30 to £720; £810 by £36 to £990; £1,032 by £42 to £1,116 per annum, plus 10 per cent contract addition and placing in accordance with qualifications and experience. Children's allowances (£300 maximum). Passages on appointment, annual leave and normal termination. Gratuity payable in lieu of pension; overseas allowance (£240 to £270 per annum) in appropriate cases. Part-furnished accommodation at reasonable rent.

Detailed applications (six copies), naming three referees, by October 30, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF SHEFFIELD

Applications are invited for a post of Assistant Lecturer or Lecturer in Genetics to begin duties as soon as possible. Salary scales: Assistant Lecturer, £800 by £50 to £950; Lecturer, £1,050 by £50 to £1,400 by £75 to £1,850. Commencing salary according to qualifications and experience with F.S.S.U. provision and family allowance. A grant towards removal expenses will be made.

Further particulars may be obtained from the Registrar, to whom applications (three copies), should be sent by October 14, 1961.

NATIONAL RESEARCH COUNCIL OF CANADA

requires

MATHEMATICIANS

for its

COMPUTATION CENTRE

Work will involve the analysis and programming of problems which arise in the various scientific and engineering divisions of the Council. The Council has recently acquired an I.B.M. 1620 computer with card and paper tape input-output facilities and a core memory of 40,000 decimal digits.

Applicants should possess at least an honours degree in mathematics. Previous experience with computers is desirable but not essential.

Salary up to $8,700 per annum depending on education and experience.

Applications should be made to the Employment Officer, National Research Council, 100 Sussex Drive, Ottawa, Ontario.

In reply, quote file E.R.-210.

GOVERNMENT OF THE FEDERATION OF RHODESIA AND NYASALAND

FEDERAL MINISTRY OF AGRICULTURE

Due to the further expansion of the Agricultural Services, applications are invited for the following vacancies, the majority of which are in Southern Rhodesia:

AGRONOMISTS
Two posts at Research Stations.

APICULTURISTS
Research and Specialist Services Branch.

SYSTEMATIC BOTANIST
Government Herbarium, Salisbury.

SEEDS RESEARCH OFFICER
Seed Laboratory, Salisbury.

CROP INSPECTOR
Research and Specialist Services Branch.

WEED OFFICER
Botany and Plant Pathology Branch, Salisbury.

Applicants for posts in this group will be graduates and starting salaries will be between £752 10s. and £1,397 10s. per annum, depending on qualifications and experience, rising on a scale to £1,773 15s. per annum.

CURATOR, Botanical Gardens, Salisbury.

Applicants will have a diploma or equivalent qualification recognized internationally. Practical experience will be an advantage. Commencing salary will be £1,161 rising on a scale to £1,505 per annum.

Family passages, pensions, medical aid, holiday grants, generous sick, occasional leave, car advances, travelling and field allowances, good schools—free tuition, opportunities sport and recreation. Low income tax, e.g., married man (two children), £1,400, tax £15; married man (two children), £2,500, tax £212; plus territorial surcharge 20 per cent.

Application forms and further details (state post) from:

The Secretary (R), Rhodesia House, 429 Strand, London, W.C.2.

Closing date: October 20, 1961.

UNIVERSITY OF MELBOURNE

Applications are invited for a position of Lecturer in Pure Mathematics at the R.A.A.F. Academy, which is affiliated with the University of Melbourne. The Lecturer will be a full-time officer of the university, and his work will be subject to the general direction of the Professor of Mathematics (R.A.A.F. Academy) Professor B. C. Rennie. The lecturer will be expected to engage in research which will be promoted or pursued in either the University or the Academy or in both places if appropriate. The salary range is £A.1,660 to £A.2,360 per annum. Initial salary will be determined according to qualifications. Superannuation similar to F.S.S.U. in Great Britain will be provided.

Conditions of appointment may be obtained from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on October 31, 1961.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

PLANT PHYSIOLOGIST

The Organization's Fodder Conservation Section, Melbourne, Victoria, invites applications for a position as Plant Physiologist from honours graduates in science or agricultural science (or equivalent qualifications) with relevant postgraduate research experience in plant physiology. For appointment at the senior level applicants should have an established reputation in research.

Fodder conservation is of a major importance to the agricultural economy of Australia, not only in southern districts but also in the North where the full potential of recent developments in pasture improvement can be realized only through efficient conservation. The Section has a team of research officers engaged in various fields of study of fundamental importance in problems of fodder conservation. One of the more promising of these now being developed is the study of post-harvest physiological changes in pasture plants either during drying (as in haymaking) or under anaerobic conditions (as in ensilage).

The appointee will have the opportunity for a wide range of fundamental work on the physiology of pasture plants including collaborative work with the Section's biochemists.

SALARY: Dependent on qualifications and experience within the ranges, £A.2,426 to £A.2,751 per annum or £A.3,011 to £A.3,531 per annum.

Salary for a woman will be £A.188 per annum less than corresponding rates for men.

By mutual arrangement, the appointment may either be for an indefinite period or for a fixed term of three years.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid and for a short term contract return fares will be paid. Further particulars of conditions, etc., supplied on application to:

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No.: 400/93), should be addressed by October 28, 1961.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

RESEARCH OFFICER OR SENIOR RESEARCH OFFICER —ANIMAL PRODUCTION

The Organization's Division of Plant Industry, Canberra, A.C.T., invites applications for a research appointment in an expanding research group to study the utilization and improvement of pastures. Particular emphasis is to be placed on the interactions between plants and grazing animals and their effects on animal nutrition and physiology, and on the output of animal products. It is thus envisaged that these studies will include both basic and applied fields of animal research.

The appointee will be a member of the Division's newly formed Grassland Agronomy Section. In these laboratories there are over 70 research officers engaged in research in many fields of plant and animal production and biology, extending from agronomy, ecology and microclimatology to genetics, physiology and biochemistry. The new Section has established close working relationships with many of these teams. There is an excellent library and a wide range of scientific equipment. There is a Field Station of 2,400 acres and extensive facilities are being developed for field and animal house research.

Applicants should be honours graduates in Science, Agricultural or Rural Science, or Veterinary Science and should have relevant postgraduate research experience and competence in some branch or branches of animal research which are relevant to pasture production.

SALARY: Dependent on qualifications and experience within the ranges:
£A.1,541 to £A.2,296 per annum or £A.2,426 to £2,751 per annum.

Salary for a woman will be £A.188 per annum less than corresponding rates for men.

By arrangement, the appointment may be for an indefinite period or for a fixed term of three years.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid and for a fixed term contract, return fares will be paid. Further particulars of conditions, etc., supplied on application to:

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No.: 130/504), should be addressed by October 28, 1961.

XUM

NATIONAL RESEARCH COUNCIL OF CANADA

invites applications from

PHYSICISTS

for its

DIVISION OF APPLIED PHYSICS

for research in the following fields:

Acoustics, temperature measurement, plasmas, thermoelectrical conductivity at high temperatures (including ceramic materials), beta ray spectroscopy, nuclear physics, optical masers, photon counting methods for photometry, optical image evaluation, applications of physics to survey science, the development of instruments, mechanical measurements, and electrical standards (all frequencies).

Demonstrated research ability more important than previous field of experience for younger applicants. Very favourable conditions in respect of research freedom, apparatus, attendance at scientific meetings, etc.

Salary range $5,000 to $12,000, depending on qualifications and experience. Starting rate for fresh Ph.D., $7,050. Regular annual increments, accelerated promotion for first-class man.

Inquiries and applications should be directed to the

Director of the Division of Applied Physics,
National Research Council, Sussex Drive, Ottawa 2, Canada.

Please quote File AP-149.

SHELL **Shell Chemicals**

STATISTICS IN RESEARCH AND DEVELOPMENT

Carrington Research Laboratory (near Manchester) has a vacancy for a graduate interested in the application of statistical methods in all phases of its operations. The work covers the design of experiments and the interpretation of results, the optimization of process conditions and the control of chemical, physical and mechanical testing procedures.

Men or women candidates should be under 30 years of age and have a degree in:

either **(a) CHEMISTRY or CHEMICAL ENGINEERING and research experience in these fields, with a subsequent qualification in statistics or experience in the use of statistical methods.**

or **(b) MATHEMATICS or STATISTICS with a real interest in the application of statistical methods in research and production.**

Salary based on qualifications and experience. Excellent Pension Scheme, and other benefits.

Applications (quoting Ref. CRL.134), to:

SHELL CHEMICAL COMPANY LIMITED
Personnel Department, 29 Old Burlington Street,
LONDON, W.1.

CHELSEA COLLEGE OF SCIENCE AND TECHNOLOGY

MANRESA ROAD, LONDON, S.W.3
DEPARTMENT OF CHEMISTRY

A Scientific Assistant is required in the Chemistry Department for the maintenance of physical instruments including spectrophotometers and radiochemistry equipment. Duties will also include certain routine measurements and demonstrations. The person appointed will be directly responsible to the Head of the Department. Salary scale: £940 by £40 to £1,100 per annum.

Further details of the appointment available on application. Closing date for applications: October 21, 1961.

BATTERSEA COLLEGE OF TECHNOLOGY

LONDON, S.W.11

Applications are invited from graduates of a British University for the post of Lecturer in Physiology in the Department of Health Education. Candidates should have had teaching experience and, preferably, should be qualified to undertake some teaching of biology. The successful candidate will be encouraged to undertake research for which appropriate relaxation will be granted. Salary £1,408 by £35 to £1,601.

Further particulars from the Clerk to the Governing Body, to whom applications should be sent as soon as possible.

PROTOZOOLOGIST

Required by the West African Institute for Trypanosomiasis Research at Vom, Northern Nigeria. A wide field of research is open according to previous experience and ability. Appointment will be on contract for two tours of 12 to 24 months in the first instance. Salary according to qualifications and experience in scale £1,248 a year rising to £2,820 a year. Gratuity at rate of £150 a year payable at end of a tour or on final completion of service. Free passages for officer and wife. Assistance towards children's passages and/or grant up to £150 a year if educated in U.K. Liberal leave on full salary. Quarters provided at moderate rental. Candidates must possess a good honours degree in zoology or biology and have had not less than two years postgraduate research experience in protozoology or parasitology. Experience of trypanosomiasis work, or modern techniques in immunology, or the use of radioactive tracers would be an advantage. Women candidates must be single.

Apply to Crown Agents, 4 Millbank, London, S.W.1, for application form and further particulars, stating age, name, brief details of qualifications and experience and quoting reference M3B/53573/NF.

UNIVERSITY OF QUEENSLAND

READER AND SENIOR LECTURER IN ANATOMY

The University invites applications for the above-mentioned positions.

Applicants for the Readership should be well qualified in the subject, have had considerable experience of teaching at University level, hold a higher degree, preferably a doctorate, and have made significant original contributions to some branch or branches of Anatomy, or to related subjects such as Neurophysiology, Histopathology, etc. It is preferable that the appointee be medically qualified.

Applicants for the Senior Lectureship should have had a sound training in the subject, have had experience in teaching at University level, hold a higher degree and/or have made original contributions to some branch or branches of the subject, or to related subjects. It is preferable that the appointee be medically qualified.

The Department of Anatomy will from the beginning of 1962 occupy its new building at the main University site at St. Lucia where there will be very good facilities for teaching and for investigation. The department has its own "Akashi" electron microscope in operation and the University will shortly have a Siemens and Halske Elmiskop I installed in the general Electron Microscope Unit, to which staff of the Department of Anatomy will have access. There is full provision in the Department for modern cytological and histological investigation. It is expected that there will also be adequate provision of electronic equipment.

The salary ranges are:
Reader: £A.2,960 by £A.60 to £A.3,200
Senior Lecturer: £A.2,520 by £A.70 to £A.2,870

The successful applicants will be entitled to participate in the benefits available to the academic staff which include F.S.S.U. type superannuation, Housing Assistance, Study Leave and Travel Grants.

Additional information on the conditions of appointment, staffing and activities of the Department, together with application forms, will be supplied upon request to the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

Applications close, in Australia and London, on November 11, 1961.

UNIVERSITY OF MALAYA IN SINGAPORE

Applications are invited for (A) Lectureship or (B) Assistant Lectureship in Anatomy. Salary scales: For medically qualified candidates (A) £1,295 by £49 to £1,442/£1,540 by £56 to £1,988 per annum. (B) £1,148 by £49 to £1,246 per annum. For non-medically qualified candidates: (A) £1,148 by £49 to £1,442/£1,540 by £56 to £1,820 per annum. (B) £1,001 by £49 to £1,099 per annum, entry point determined by qualifications and experience. Allowances: expatriation in range £280 to £308 per annum, cost of living (temporary) in range £210 to £560 per annum. Passages for appointee, wife and children not exceeding 12 years. Partly furnished house/flat at reasonable rent. Provident Fund Scheme.

Detailed applications (eight copies), naming three referees, by October 31, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

XUM

UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND

Applications are invited for (A) Lectureship or (B) Assistant Lectureship in Mathematics. Salary scales: (A) Grade I, £1,600 by £50 to £1,900 per annum. Grade II, £1,350 by £50 to £1,600 per annum. (B) £1,200 by £50 to £1,350 per annum. Passages to Salisbury for appointee, wife and dependent children on appointment, with allowance for transport of effects. Superannuation and Medical Aid Schemes. Unfurnished accommodation at rent of 15 per cent of salary. Assistance given for triennial visits overseas.

Detailed applications (six copies), naming three referees, by November 5, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

WIGGINS TEAPE

Applications are invited for the following post at the Group's Mill near Aberdeen.

CHEMICAL ANALYST

to head a Chemical Service Section.

The work will involve supervising, co-ordinating and participating with a small staff engaged in chemical investigation and examination of all papermaking materials and products.

Applicants must have appropriate Laboratory experience, first-class judgment and an appropriate degree and/or A.R.I.C.

Training and introduction to the work by the retiring predecessor will be given to the selected candidate.

Please write in first instance, quoting reference CA, to:

THE WIGGINS TEAPE GROUP,
Staff Training Centre,
Butler's Court, Beaconsfield, Bucks.

PHILIPS RESEARCH LABORATORIES

Philips Research Laboratories in Eindhoven, Holland, require an Editor for the English edition of the *Philips Technical Review*—a scientific journal with a world-wide circulation. Candidates should have several years' research experience and preferably a higher degree in physics, chemistry or electrical engineering. Knowledge of Dutch not essential.

The successful candidate will be a full member of the Research Laboratory with facilities for undertaking research in addition to his editorial duties. The Philips Research Laboratory in Eindhoven is the principal research centre of an international federation of companies which employs over 200,000 people, including several thousand scientists engaged on research and development. The post involves regular contact with the Philips companies in this country. Assistance will be provided with housing.

Applications, clearly marked Ref. ABC, should be addressed in the first instance, to the Chief Personnel Officer, Philips Electrical Industries Limited, Berkshire House, High Holborn, W.C.1.

UNIVERSITY OF READING. APPLICATIONS are invited for two Lectureships in Food Science from persons with special qualifications and experience in the food industry (other than the dairy industry) or in biochemical engineering. The Lecturers will be required to undertake the teaching of food technology in a newly-introduced degree course.—Further particulars may be obtained from the Registrar (Room 22 O.R.B.), the University, Reading, by whom applications must be received not later than October 31, 1961.

Return to research in Britain

Are you a British scientist in Canada thinking of returning to research in Britain? You may find what you are looking for, without having to go home first, in the Civil Service or the United Kingdom Atomic Energy Authority.

Research Fellowships and Permanent Appointments

You are offered a very wide choice, in all branches of science and engineering, at a large number of centres where research ranges from the most fundamental to the most applied.

A panel of scientists will be coming to Ottawa on 17th January 1962

If you would like to meet them, write by 15th November, 1961, to:

Mr. N. R. Geary, (34)
U.K.A.E.A. Representative,
c/o Atomic Energy of Canada, Ltd.
Chalk River, Ontario

An announcement to British scientists in the U.S.A. will be made in December

Issued jointly by the Civil Service Commission and the United Kingdom Atomic Energy Authority

UNIVERSITY OF NEW ENGLAND
ARMIDALE, NEW SOUTH WALES
LECTURER OR SENIOR LECTURER IN PHYSIOLOGY

Applications are invited for the abovementioned position. The Department of Physiology, which is at present expanding, offers courses in two faculties and within the Faculty of Science teaches to honours and postgraduate level. An applicant with general or specific training in physiology and qualifications in either science, veterinary science, medicine or agricultural science will be considered for the position. The Department is, however, particularly interested in developing its courses in general and comparative mammalian physiology and the biophysical aspects of physiology. The successful applicant will be expected to assist in the teaching of second and third year students in the Faculty of Science and to continue his own line of research or to join one of the research projects already in progress. Salary for a Lecturer is within the range £A.1,730 by £A.105 to £A.2,435 and for a Senior Lecturer £A.2,550 by £A.100 to £A.3,000 per annum. In addition cost of living adjustments at present amount to £A.49. The salary for overseas applicants commences from the date of embarkation and fares of applicant and family are paid, together with a removal allowance of £A.300. The commencing salary will be fixed according to qualifications and experience. The position is superannuable and up to 90 per cent of the purchase price of a house may be advanced through the university housing scheme.

Further information and the conditions of appointment may be obtained from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on November 30, 1961.

POLYMER RESEARCH CHEMIST

A Polymer Research Chemist is sought for new Research Laboratories at present being established in a country estate in South Oxfordshire. Suitable applicants are expected to be under 35 years, and to have research interests and experience. Either an academic or an industrial research background would be considered suitable.

The position will interest those with a sound general knowledge of polymer chemistry, who are anxious to find scope and full support for developing their own ideas in an unusually wide field. Experience in ion-exchange would be an advantage, but is not essential. Publication of results will be encouraged.

Contributory superannuation scheme, removal expenses, and assistance with rehousing, are provided. Send details of interests, research achievements, and qualifications, to:

The Director of Research,
AMF Research Centre, Blounts Court,
Sonning Common, Reading.

NEWCASTLE REGIONAL HOSPITAL BOARD
PHYSICIST—SENIOR GRADE

Applications are igvited for the post of Senior Physicist to the Newcastle Regional Hospital Board. The candidate appointed will have responsible duties in the Megavoltage Institute at the Newcastle General Hospital, which is equipped with two linear accelerators, cobalt and caesium units, as well as some duties at regional hospitals. Experience of high energy therapy, preferably at megavoltage level is desired. Opportunities will be offered for research in the expanding work of the department, for which new headquarters and laboratories are about to be built on an adjoining site. Salary will be in accordance with the Whitley Council Senior scale, e.g., £1,250 rising by annual increments to £1,700.

Applications, accompanied by the names and addresses of two persons to whom reference may be made, should be sent to the Secretary, Newcastle Regional Hospital Board, Benfield Road, Newcastle upon Tyne 6, as soon as possible.

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)

A vacancy exists in the Department of Chemistry for postgraduate or postdoctoral work on some fundamental aspects of the electrochemistry of solutions. The work will be suitable for a higher degree. Salary in the range £500 to £650 per annum, plus fees for the postgraduate, and from £800 per annum for the post-doctoral appointment.

Further particulars should be obtained from the Secretary, Birkbeck College, Malet Street, London, W.C.1. Closing date: October 16.

WELSH COLLEGE OF ADVANCED TECHNOLOGY
DEPARTMENT OF ENGINEERING
RESEARCH STUDENTSHIP

A Research Studentship for one year, with possible renewal to give tenure of three years, is available in the Department of Engineering. The holder will be required to pursue research whether this is intended to lead to a higher degree or not. The salary payable will be £550 per annum, increasing by annual increments of £50 to £650 per annum. The successful applicant may be allowed to undertake up to six hours teaching a week for which payment would be made.

Applications, giving details of qualifications and experience to date, as well as an outline of fields of interest or research work proposed, should be sent to the Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff, as soon as possible.

UNITED KINGDOM ATOMIC ENERGY AUTHORITY
require

PHYSICISTS OR ENGINEERS
for training in the field of

NUCLEAR REACTOR OPERATION

The successful applicants will be expected to familiarize themselves with the operation of the Reactors and associated Power Plant at Calder Hall Power Station, and will assist on a variety of investigations and projects aimed at increasing the efficiency of the plant. The appointments will be initially on days but there is the prospect of promotion to Shift Manager of the station when a vacancy arises if the qualities required for this are shown.

Applicants should have a good honours degree in Physics or Engineering or corporate membership of the Institute of Electrical or Mechanical Engineering, and have some experience in industrial plant operation or research and development work.

The post is superannuable and the successful applicant will be eligible for housing or assistance with house purchase.

Salary £1,005 (at age 25) rising by annual increments to £1,535 per annum.

Applications, quoting Ref. P/W. 121/J.14, should be made to: Staff Appointments Officer, Windscale and Calder Works, Sellafield, Seascale, Cumberland.

Closing Date: October 16, 1961.

BIOCHEMIST

Required by the West African Institute for Trypanosomiasis Research at Kaduna or Vom, N. Nigeria, for Biochemical Research related to any aspect of Trypanosomiasis. Appointment will be on contract for two tours of 12 to 24 months in the first instance. Salary according to qualifications and experience in scale £1,248 a year rising to £2,820 a year. Gratuity at rate of £150 a year payable at end of a tour or on final completion of service. Free passages for officer and wife. Assistance towards children's passages and/or grant up to £150 a year if educated in U.K. Liberal leave on full salary. Quarters provided at moderate rental. Candidates must possess a good honours degree in Biochemistry or Chemistry, or a registrable medical degree, with additional qualification in biochemistry, and have had at least two years' postgraduate research experience in biochemistry. Women candidates must be single.

Apply to Crown Agents, 4 Millbank, London, S.W.1, for application form and further particulars, stating age, name, brief details of qualifications and experience and quoting reference M3B/53575/NP.

SOUTH-WEST ESSEX TECHNICAL COLLEGE
Forest Road, Walthamstow, E.17

Required as soon as possible: (1) Lecturer in Inorganic Chemistry to teach B.Sc. General, H.N.C., and Grad.R.I.C. students. Candidates should be graduates, and a higher degree and/or specialization in a branch of chemistry would be an advantage. Required January 1, 1962. (2) Assistant Lecturer (Grade B) to teach Physics to G.C.E. Advanced Level, with some B.Sc. General Part I work. Candidates should be graduates, preferably with a higher degree and/or evidence of specialization in a branch of physics. In respect of these posts research will be encouraged. Salaries within scales: Lecturer, £1,370 to £1,550 per annum: Grade B £700 to £1,150 per annum (both plus London allowance of £38 or £51 per annum). Grade B scale subject to additions for approved training and graduate qualifications, and the commencing salary within this scale will have regard to War, National Service and previous experience.

Apply by letter (no forms) to the Principal, giving details of training, qualifications and experience, together with three recent testimonials, or the names and addresses of three referees.

UNIVERSITY OF MANCHESTER

Applications are invited for appointment as Spectroscopist in the Department of Chemistry. The successful candidate would have general responsibility for the maintenance and use of a range of spectroscopic equipment in the Department, including N.M.R., infrared, Raman and U.V. He would be expected to collaborate with members of the teaching staff on research problems and to supervise routine measurements required. Candidates should preferably have a degree in physics or chemistry and should have experience in spectroscopy. Appointment would be in the grade of Experimental Officer or Senior Experimental Officer. Salary range: £700 by £50 to £790 by £40 to £1,000 per annum (Experimental Officer); and £1,000 by £50 to £1,250 (more bar-line) by £50 to £1,500 (Senior Experimental Officer). The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 9, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

NATIONAL COLLEGE OF FOOD TECHNOLOGY
ST. GEORGE'S AVENUE,
WEYBRIDGE, SURREY
Principal: J. D. Mounfield, M.Sc.Tech., Ph.D., F.R.I.C.

Applications are invited for the post of Assistant Lecturer in Chemistry to the College. Facilities for research are available. Salary scale £790 by fifteen at £27 10s. and one at £37 10s. to £1,240 per annum. Starting salary dependent on qualifications and experience.

Application forms (obtainable from the College) should be sent to the Principal as soon as possible.

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
(UNIVERSITY OF READING)

Applications are invited from honours graduates in physics to take part in a programme of work concerning the physical properties of milk. Preference will be given to candidates who have had appropriate research experience. Salary within range £1,342 to £1,654 with F.S.S.U. pension provision.

Applications, naming two referees, to the Secretary, N.I.R.D., Shinfield, Reading. Quote reference 61/27.

APPLICATIONS ARE INVITED FROM male laboratory technicians for a post of *Senior Assistant (Scientific)* in the Medical Branch of H.M. Factory Inspectorate. The work involves chemical and clinical pathology in the study and diagnosis of industrial diseases. A small but well-equipped laboratory is in London. Opportunities for travel may occur. Applicants should possess the final certificate in chemical pathology of the I.M.L.T. *Salary scale:* £866 to £1,142. The successful candidate will be considered for establishment to non-contributory pensionable post within 12 months of appointment. 5-day week; 3 weeks' annual leave plus public and privilege holidays.—Apply in writing within seven days to Establishment Officer, Ministry of Labour, O.E.II, 19 St. James's Square, London, S.W.1.

ROYAL COLLEGE OF ADVANCED TECHNOLOGY

SALFORD

RESEARCH FELLOWSHIPS

Applications are invited for two Research Fellowships to be held in the Department of Mechanical Engineering. The Research Fellows will be expected to carry out research full-time in the Department in one of the following fields: Vibration phenomena in machine tools. Control systems. Friction and lubrication, including the performance of bearings. Forming and machining processes. The facilities for research are good and a programme of research in machine tools is at present supported financially by D.S.I.R. Applicants should have had postgraduate research or other appropriate experience in a relevant field. Appointments will be made within the salary range £700 rising to £1,150, the commencing salary depending upon qualifications and experience.

Further particulars and forms of application may be obtained from the Registrar, the Royal College of Advanced Technology, Salford 5, Lancs., to whom completed forms should be submitted.

UNIVERSITY OF MANCHESTER

Applications are invited for the appointment as Experimental Officer or Senior Experimental Officer in the Department of Mechanics of fluids. His duties will include the design and construction of electronic and optical apparatus for studying turbulence, aerodynamic noise and flows at very high speeds including their interaction with magnetic fields. Salary range: £700 by £30 to £790 by £40 to £1,000 per annum (Experimental Officer); and £1,000 by £50 to £1,250 (merit bar-line) by £50 to £1,500 (Senior Experimental Officer). The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 23, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

MAKERERE UNIVERSITY COLLEGE

EAST AFRICA

Applications are invited for appointment to the Chair of Physics. Salary £3,000 per annum. F.S.S.U. Child allowance £50 per annum per child (maximum £150 per annum). Passages for appointee and family (up to five adult passages) on appointment, termination and leave (three months every 21 months). Rent according to quarters provided £45 to £84 per annum (including basic furniture).

Detailed applications (eight copies), naming three referees, by November 10, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

GOVERNMENT OF NORTHERN IRELAND

SCIENTIFIC OFFICER

Applications invited for pensionable post in Chemical Research Division, Ministry of Agriculture. Qualifications: Honours degree in chemistry or agricultural chemistry. Successful candidate will be employed mainly on forestry research work and may at some future date be required to undertake teaching duties in Faculty of Agriculture, Queen's University, Belfast. Salary scale £738 to £1,222; a candidate with special qualifications, training or experience may enter above minimum. Preference for ex-Servicemen.

Application forms obtainable from Secretary, Civil Service Commission, Stormont, Belfast, 4, must be returned by October 18, 1961.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Director of the Occupational Hygiene Unit in the Department of Occupational Health from graduates in chemistry, physics or engineering. The object of the Unit (which is financed by a grant from the Nuffield Foundation) is to provide an Occupational Hygiene Service for the industry of the North-west of England on a fee paying basis. It will investigate the demand for such a service and its cost. Duties to commence on January 1, 1962. Initial salary £2,250 per annum with membership of the F.S.S.U. and Children's Allowance Scheme.

Forms of application and further particulars may be obtained from the Registrar, the University, Manchester, 13, to whom applications should be sent not later than October 2, 1961.

UNIVERSITY OF QUEENSLAND

RESEARCH FELLOW OR SENIOR RESEARCH FELLOW IN PARASITOLOGY

Applications are invited for the above-mentioned position in the Department of Parasitology in the University of Queensland. The duties will include the instigation of research work on pathogenesis of parasitic infections in domestic animals; a certain amount of undergraduate teaching will also be required. The tenure of the Fellowship will be three years.

Applicants should have a degree in Veterinary Science and postgraduate research experience. Salary will be in the range £A.1,500 to £A.2,200 depending upon qualifications and research experience. The higher salary range will only be paid if the applicant possesses considerable research experience.

Applications should be forwarded to the Registrar, University of Queensland, Brisbane, Queensland, Australia, not later than October 28. Further information will be supplied upon request.

C. J. CONNELL,
Registrar.

BRITISH GUIANA

GEOLOGIST

Qualifications: First- or good second-class honours degree in Geology or Mining Geology. Experience desirable.

Duties: General geological mapping and mineral investigations in the interior. Work mainly on precambrian crystalline rocks, with emphasis on petrographical, structural and economic studies.

Terms of appointment: On contract for tour of two years with salary according to experience in scale £950 to £1,400 per annum plus gratuity of 22½ per cent. Outfit allowance. Free family passages. Government quarters at rental if available. Generous leave. N.B.: These terms are likely to be improved when details of the application of the Overseas Service Aid Scheme to British Guiana have been worked out, they will, however, only be granted to officers designated under the Scheme. Generally, a candidate for appointment who was born in and whose permanent home is in the United Kingdom will qualify for designation.

Apply to Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting C1/RC.218/16/01, and stating full name, age, qualifications and experience.

UNIVERSITY OF MANCHESTER

Applications are invited for appointment of an Experimental Officer or Senior Experimental Officer to assume responsibility for supervision and maintenance of electronic control computer and digital equipment associated with the Radio Telescope and other apparatus at Jodrell Bank. Salary scales attached to this post are: £700 by £30 to £790 by £40 to £1,000 per annum (Experimental Officer); and £1,000 by £50 to £1,250 (merit bar-line) by £50 to £1,500 (Senior Experimental Officer). The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than October 7, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

A QUALIFIED CHEMIST OR PHYSICAL CHEMIST

is required by The Carborundum Company Limited. Minimum qualifications H.N.C. in chemistry, physics or applied physics. A background in Spectrography or X-ray Diffraction will be of advantage. The work concerns the development of techniques and application of physico-chemical methods of raw materials and product testing. The post offers good prospects, excellent working conditions, bonus scheme and a generous contributory pension scheme.

All applications, which will be treated in strict confidence, should include full details of age, qualifications and experience and should be addressed to the Personnel Office, The Carborundum Company Limited, Trafford Park, Manchester 17.

MIDDLESEX COUNTY COUNCIL EDUCATION COMMITTEE

BRUNEL COLLEGE OF TECHNOLOGY
WOODLANDS AVENUE, ACTON, W.3
Principal: J. Topping, M.Sc., Ph.D., D.I.C., F.Inst.P.

RESEARCH ASSISTANT—MATHEMATICS DEPARTMENT

There is a vacancy for a Research Assistant in the Mathematics Department. A National-Elliott 803 Digital Computer will shortly be installed. The Research Assistant will be responsible for the day-to-day administration of the computer. He will be expected to assist potential users, both from within the College and from outside, to formulate and programme their problems. He should have experience of numerical analysis and of high-speed computers. There are no formal teaching duties attached to this post. The successful candidate will be encouraged to study for the M.C.T. or higher degree should he so desire.

Salary on scale comparable with Assistant Lecturer, Grade B (£826 to £1,496) per annum.

Applications to the Registrar at the College.

C. E. GURR, M.Sc., Ph.D.,
Chief Education Officer.

MIDDLESEX COUNTY COUNCIL EDUCATION DEPARTMENT

Required at Isleworth Polytechnic:

(a) LABORATORY STEWARDS for duties in new machine shops, Engineering, Electronics and Physics and Chemistry Laboratories. Salary: Misc. IV, £665 to £725 if 26 years or over.

(b) LABORATORY ASSISTANTS for duties in Engineering and Biology Laboratories. Salary: Misc. III, £595 to £665 if 26 years or over.

Both posts: Prescribed conditions. Application forms from Principal, London Road, Isleworth, returnable by October 13. (Quote H.452 N.)

LONG-RANGE INDUSTRIAL RESEARCH

There are openings for Scientists able to make original contributions to fields such as physical properties of materials, automatic control systems, applications of digital techniques. Publication of results will be encouraged. Contributory superannuation scheme, removal expenses and assistance with rehousing provided.

Send details of interests, research achievements, and qualifications, to:

The Director of Research,
AMF Research Centre, Blounts Court,
Sonning Common, Reading.

UNIVERSITY OF NATAL

DEPARTMENT OF MATHEMATICS AND APPLIED MATHEMATICS

Applications are invited from suitably qualified persons for appointment to the post of

PROFESSOR OF MATHEMATICS OR APPLIED MATHEMATICS, Durban

The salary attached to the post is R.5,400 (£2,700) per annum, fixed.

Applicants who have specialized in Pure or Applied Mathematics or in Theoretical Physics will be considered on the same basis, no one of these disciplines being given preference over the other.

Application forms, and further particulars of the post, including commencing dates, etc., are obtainable from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

Applications close, in South Africa and London, on October 31, 1961.

ASSISTANT EXPERIMENTAL OFFICER OR Experimental Officer required by War Office, Directorate of Civilian Medical Services, Central London. Duties: work in close co-operation with medical officer (radiation), and site and personnel monitoring. Expected to keep abreast of current developments and literature. Opportunities for applied research. Selected candidate will attend training courses. Qualifications: Pass degree or H.N.C. in physics or applied physics. Good knowledge of health physics and/or chemistry. Age, Assistant Experimental Officer, 18 to 28. Experimental Officer, minimum age 27. Salary: Assistant Experimental Officer, £503 to £1,038 per annum. Experimental Officer, £1,147 to £1,396 per annum. Opportunity for establishment and promotion.—Forms from Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting A 376/1A.

UNIVERSITY OF QUEENSLAND

The University invites applications for the following positions:

LECTURER IN BOTANY

The appointee should have special training and interest in research in plant physiology, preferably in cell physiology or metabolism, but also a sound knowledge of the general field of Botany. Some knowledge of the flora of Australia or New Zealand is desirable.

He will be expected to give lectures and supervise practical work on plant physiology in conjunction with the courses given by the senior lecturer in plant physiology to students in the Faculties of Science and Agriculture, and to deliver lectures in such other aspects of Botany as lie within his general interests. It is expected that he will carry out research in his chosen field.

LECTURER IN GEOGRAPHY

Applicants should hold an honours degree in Geography and should have a special interest in one of the following fields—economic Geography, regional development or the Regional Geography of North America or Asia.

The Lecturer will be required to lecture to internal students in one of above areas of work and, as from 1963, to provide written material for and supervise the work of second-year students in economic and regional geography.

SENIOR LECTURER IN MATHEMATICS

Applicants should have an honours degree in Mathematics, and preferably a Higher Degree, and some experience of research. The applicant's special field of interest may be in Pure Mathematics, Applied Mathematics or Mathematical Statistics.

The Department of Mathematics is responsible for the teaching of Pure and Applied Mathematics and Statistics within the University. Basic courses in Mathematics are common to several faculties; a degree with honours in Mathematics is available. The University has recently planned a Computer Centre and a GE 225 Computer is expected to be in operation in 1962.

LECTURER IN PHYSICS
and
LECTURER IN THEORETICAL PHYSICS

Applicants should possess an honours or higher degree in Physics. Preference will be given to applicants with previous teaching and research experience.

The Department of Physics is engaged in the following fields of research; Electrical Properties of the Upper Atmosphere (Ionosphere and Exosphere); Electro-magnetic phenomena of extra-terrestrial origin; Micro-wave properties of gases and plasmas; Determinations of atomic abundances in stellar atmospheres (theoretical only): Applications of ionizing radiations in medicine.

The salary ranges are:
Senior Lecturer: £A.2,520 by £A.70 to £A.2,870
Lecturer: £A.1,830 by £A.70 to £A.2,330

The successful applicants will be entitled to participate in the benefits available to the academic staff which include F.S.S.U. type Superannuation, Housing Assistance, Study Leave and Travel Grants.

Additional information on the conditions of appointment, staffing and activities of the Departments, together with application forms, will be supplied on request to the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

Applications close, in Australia and London, on October 31, 1961.

RADIOLOGICAL PROTECTION SERVICE (Ministry of Health and Medical Research Council), Clifton Avenue, Belmont, Sutton, Surrey, requires the following staff:

JUNIOR TECHNICAL OFFICER to assist Physicist developing techniques for measuring low doses of ionizing radiations and investigating miscellaneous radiation protection problems. Minimum qualifications: Two appropriate subjects at G.C.E. "A" level or O.N.C. Salary: According to age and experience at a point on the scale £410 to £860 per annum plus London weighting. Five-day week. Eighteen days leave per year.

JUNIOR TECHNICIAN to assist in the Film Monitoring Department. Applicants should hold G.C.E. Ordinary Level in at least four subjects which must include English Language, Mathematics and one Science subject, preferably Physics. Salary according to age at a point on the scale £235 per annum (age 16) to £460 per annum, plus £10 to £20 per annum London Weighting Allowance. Five-day week. Three weeks leave per year.

Applications, with the names and addresses of two referees, to the Director at the above address.

NORTHAMPTON COLLEGE OF ADVANCED TECHNOLOGY
LONDON
DEPARTMENT OF APPLIED PHYSICS

Applications are invited for the post of Technical Assistant to assist in research on Subjective Brightness. The work is being sponsored by the D.S.I.R. and the appointment is in the first place for three years. Applicants should hold a University degree or equivalent qualifications. Ability to assist in the construction of research apparatus is essential. Salary £800 per annum.

Application forms and further particulars may be obtained from the Head of the Applied Physics Department, Northampton College of Advanced Technology, St. John Street, Clerkenwell, London, E.C.1.

STUDLEY COLLEGE
WARWICKSHIRE
AGRICULTURAL AND HORTICULTURAL COLLEGE FOR WOMEN
(TWO-YEAR DIPLOMA COURSES IN HORTICULTURE AND DAIRY FARMING)

Lecturer (man or woman) required January, 1962, in Horticultural Science (Botany and Plant Pathology particularly acceptable). Salary: Burnham F.E. Scale, Assistant, Grade B, £700 by £27 10s. to £1,150 plus appropriate allowances. F.S.S.U. Residence £140.

Particulars from the Secretary.

INSTITUTE OF DISEASES OF THE CHEST
(UNIVERSITY OF LONDON)

Applications are invited from B.Sc. graduates (or from those with equivalent technical qualifications) for a post in the clinical immunology laboratory, to assist in investigations covering immunological, chemical and physiological aspects of allergic reactions to fungi, and organic dusts. Immunological experience not essential. Post will suit applicants, including newly qualified graduates, with biological or chemical training. Salary on following scale: £595 by £25 (5) to £720, plus London weighting.

Apply as soon as possible to: The Secretary, Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3.

UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

Applications are invited from recent or prospective Ph.D. graduates with interests in microbiology and biochemistry to undertake research on the bacterial degradation of algal heteropolysaccharides. The post (starting salary in the range £850 to £950) is renewable each year for a period of three years.

Applications, with the names of two referees, to Dr. K. S. Dodgson, Department of Biochemistry, University College, Newport Road, Cardiff.

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
HAMPSTEAD, N.W.3
CHEMISTRY DEPARTMENT

Applications are invited for a Research Studentship in Inorganic Chemistry. The work will be concerned with Inorganic Polymers, and will be expected to register for a higher degree.

Applications should be sent to the Professor of Chemistry, from whom further particulars may be obtained.

UNIVERSITY OF SHEFFIELD

Applications are invited for a post of Research Assistant in the Department of Genetics to work in collaboration with Professor J. A. Roper on nucleo-cytoplasmic interactions in fungi. Candidates should preferably have had research experience. Maximum initial salary £900 a year, with F.S.S.U. provision.

Applications should be sent to the Registrar, from whom further particulars may be obtained.

UNIVERSITY OF NOTTINGHAM

Applications are invited for an Assistant Lectureship in Zoology. Salary within the scale £800 by £50 to £950 according to qualifications and experience, plus membership of the Superannuation Scheme for Universities and children's allowance.

Conditions of appointment and form of application to be returned by October 14, from the Registrar.

U.S.A.
UNIVERSITY OF ALABAMA MEDICAL SCHOOL, BIRMINGHAM, ALABAMA

Laboratory Technician required for Research Laboratory specializing in lipid analysis for metabolic studies.

Applications should be addressed to J. A. Balint, M.B., M.R.C.P., Division of Gastroenterology, University of Alabama Medical Center, Birmingham 3, Alabama.

LONDON COUNTY COUNCIL
SCIENTIFIC BRANCH

Experimental Officer required in County Hall Laboratory. Duties involve research, control and development in a wide field of science. £750 to £1,140 according to age and experience. Pass degree including chemistry, or H.N.C. in chemistry essential.

Apply Medical Officer of Health (D1/N/2598/9), County Hall, S.E.1.

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
DEPARTMENT OF PHYSICS

Applications are invited for the post of Post-doctoral Research Assistant to work on High Energy Physics using the Nuclear Emulsion technique. Salary £950 to £1,500 per annum according to experience.

Applications in writing, giving details of age and experience, should reach Professor E. H. Bellamy, Department of Physics, Westfield College, N.W.3, by October 20.

INSTITUTE OF CANCER RESEARCH: Royal Cancer Hospital. Graduate required in Chester Beatty Research Institute, Fulham Road, S.W.3, for biochemical research with special reference to effects of tobacco smoking. Appointment of two to three years' duration intended to afford opportunity to study for Ph.D.—Apply, with names of two referees, to the Secretary, 33 Sumner Place, S.W.7, quoting Ref. 300/g/11.

EDITORIAL ASSISTANT REQUIRED FOR the *Biochemical Journal*. Degree in science essential; editorial experience desirable.—Apply, giving the names of two referees, to the Secretary to the Editorial Board, The Biochemical Journal, 133-135 Oxford Street, London, W.1.

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Further particulars should be obtained from the Secretary, Birkbeck College, Malet Street, London, W.C.1. Closing date for applications, October 23.

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CHAIR OF CHEMICAL PATHOLOGY

The University has promoted an Ordinance for the institution of a Chair of Chemical Pathology and invites applications for the post. The Professor will have charge of a new Department replacing the existing Department of Clinical Chemistry, and will be responsible for a Chemical Pathological service for the North-eastern Regional Hospital Board and will be Adviser to the Board in Chemical Pathology.

Further particulars should be obtained from the Secretary, the University, Aberdeen, with whom applications (sixteen copies), should be lodged not later than October 31, 1961.

LECTURES AND COURSES

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

A course of postgraduate lectures on Gas and Dust Explosions arranged by Dr. J. H. Burgoyne will be given during the week commencing Monday, November 6, 1961.

Further particulars from the Registrar, Imperial College, London, S.W.7.

UNIVERSITY COLLEGE LONDON, Gower Street, W.C.1. Completed admission application forms for the B.Sc.(Special) Degree course in Chemistry, commencing October, 1962, must reach the Tutor to Science Students by December 31, 1961 (quote TS/C). Candidates are strongly advised to apply as early as possible. —Selected applicants, who will be notified individually, will be required to attend on both Thursday and Friday, February 8 and 9, 1962, from 9 a.m. to 5 p.m., for interview and associated written tests.

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Applications are invited for a Research Studentship of the value of £330 per annum, plus fees, in the Department of Psychology, normally tenable for two or three years, from October 1, 1961.

Particulars may be obtained from the Registrar, to whom applications should be sent, in triplicate, not later than October 9, 1961.

W. D. CRAIG,
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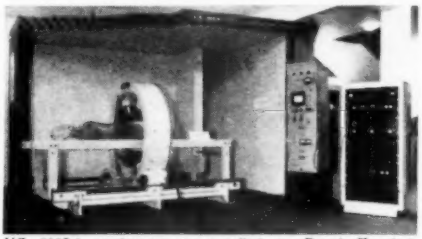

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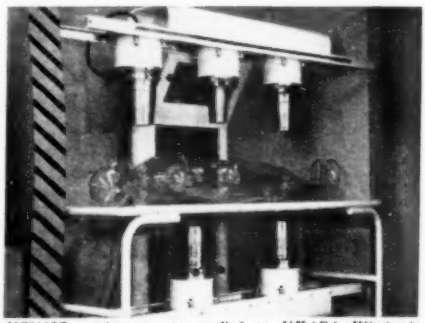

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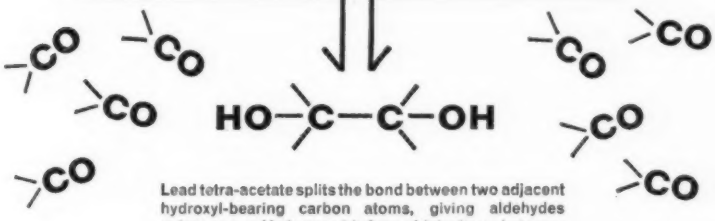

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REFERENCES: 1. *Criegee, R.*, Ber., *1931*, 64, *260* 2. *Criegee, R., Kraft, L. and Rank, B.*, Ann., *1933*, 507, *159*
3. *Grundmann, C.*, Ann., *1936*, 534. *189* 4. *Steiger, M. and Reichstein, J.*, Helv. Chim. Acta, *1936*, 19, *1016*
5. *Muller, A.*, Ber., *1934*, 67B, *830-5*

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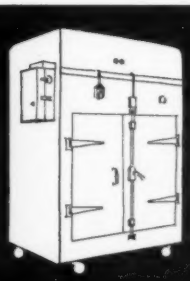

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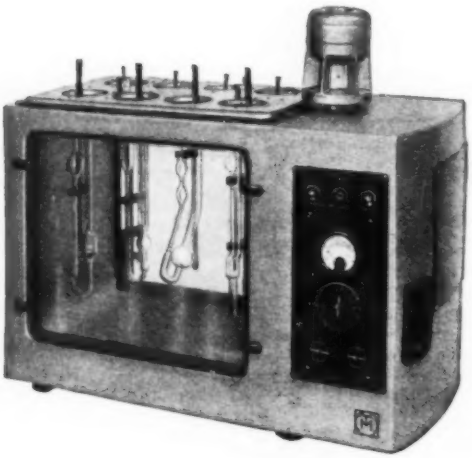

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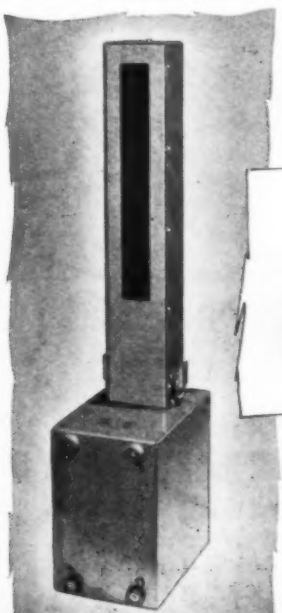

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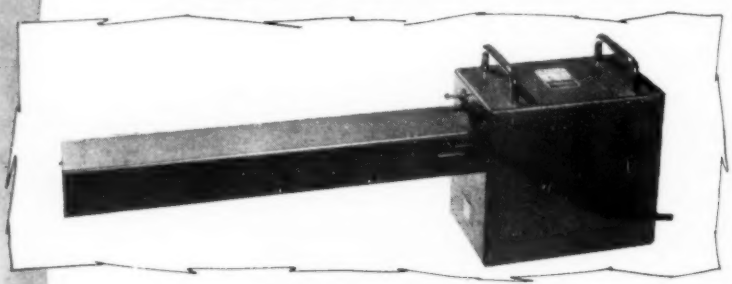

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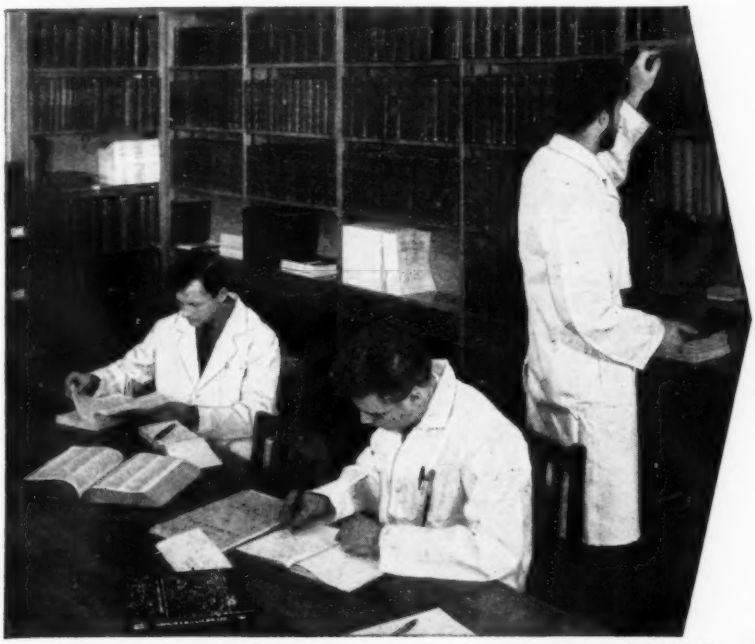

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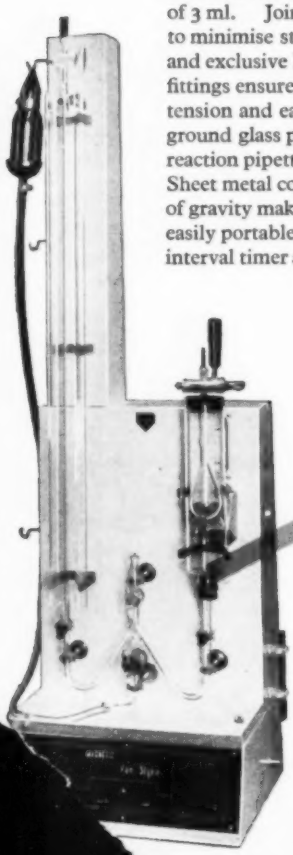

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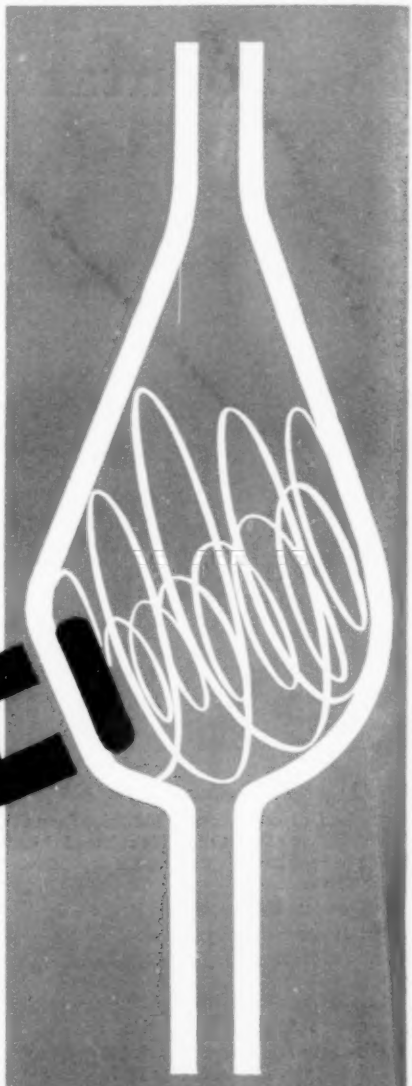

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